TAXONOMIC STUDIES OF TERRESTRIAL YELLOW-GREEN (HETEROKONTOPHYTA, XANTHOPHYCEAE) AND GREEN (CHLOROPHYTA) ALGAE FROM THE ROSS SEA REGIONS, ANTARCTICA.

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[a] Except where indicated, all are *Camera lucida* illustrations; LM, light microscopy; TEM, transmission electron microscopy; SEM, scanning electron microscopy.

ABSTRACT

Terrestrial xanthophycean and chlorophyte algae have been studied at widespread Antarctic localities. All published literature (1847 to 1998) are reviewed on chlorophyte and xanthophycean algae from terrestrial and non-marine aquatic habitats of Maritime and Continental Antarctica. A checklist of all terrestrial and freshwater algae has been compiled from all literature from 1847 to 1998. This has shown that their diversity is not fully known because of inadequacy of collections and analyses of samples. We still have insufficient base-line information about the morphological diversity of algae in Antarctica, and particularly Ross Sea regions. By detailed examination of cultured strains from the Ross Sea regions, the present study aims to contribute to the resolution of the following hypothesis: "The terrestrial algal flora of Antarctica does not comprise cosmopolitan species". The goals of the study were to: 1) provide detailed descriptions of the vegetative and reproductive characteristics of unknown, or previously poorly known, unicellular xanthophycean, and unicellular and filamentous chlorophyte algae using light and electron microscopy, 2) use isozymes to compare the genotypes of Antarctic *Botrydiopsis* and *Chlorellidium* strains with similar strains isolated from New Zealand and those in culture collections from Europe, and 3) investigate carotenoid pigments as taxonomic characters within *Botrydiopsis* and *Chlorellidium.* Each of 39 species (7 species in 3 genera from Xanthophyceae and 32 species in 14 genera from Chlorophyta) have been described and illustrated with *camera lucida* drawings and photomicrographs. There are 25 new records of chlorophyte and four new records of xanthophycean species for Antarctica. In addition, TEM has been used for the first time for 14 species. In three genera of Xanthophyceae and in *Stichococcus* TEM has revealed characteristics which are impossible to observe in sufficient detail by LM. The phenetic analysis on morphological data shows that *Botrydiopsis* and *Chlorellidium* exhibit wide morphological heterogeneity. Isozymes have not distinguished *Botrydiopsis, Botryochloris* and *Chlorellidium.* Pigment analysis has not revealed diversity at species level. Phenetic analysis of morphological data is not congruent with isozyme data and neither analysis is in agreement with the traditional system of classification. It is suggested that different techniques, e.g.18S rRNA gene sequencing should be used to examine present species concepts.

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1. A REVIEW OF THE DIVERSITY AND DISTRIBUTION OF YELLOW-GREEN AND GREEN ALGAE IN ANTARCTIC TERRESTRIAL AND NON-MARINE AQUATIC HABITATS.

1.1 Introduction

The vegetation of Antarctica is dominated by bryophytes, lichens and algae. The algal flora is dominated by cyanobacteria and chlorophytes, with xanthophyceans and diatoms as frequent associates. A recent account (Pankow *et al.,* 1991) estimated 700 taxa of non-marine algae have been recorded from Antarctica. Perhaps 200 of these occur in terrestrial habitats. Both estimates are tentative as there are major gaps in our knowledge of diversity of Antarctic algae.

The extent of global biodiversity of algae is still being debated, although there are something in the order of 12000-13000 chlorophyte and 600 xanthophycean taxa described (Andersen, 1992; Norton *et al.,* 1996). However, most phycologists suggest that the total number of algal species is from 1.2 to 10 times those presently known. The current species concept is thought by many to be too broad (e.g. Theriot, 1992; Mann and Droop, 1996), resulting in the need to describe new species. Also, many habitats, including those in Antarctica, have been insufficiently explored.

The distinction between aquatic (Vincent and James, 1996) and terrestrial habitats is not clear and undoubtedly some overlap occurs in species distribution. Here algae regarded as terrestrial are those which occur in situations ranging from the extremely wet, e.g. on and among bryophytes bordering streams, to the extremely dry, e.g. in soils of the dry valleys of southern Victoria Land (Broady, 1996).

There have been several reviews of terrestrial algae worldwide (e.g. Metting, 1981; Hoffman, 1989; Nienow, 1996) and of those in Antarctica (e.g. Broady, 1996; Nienow and Friedmann, 1993). All these accounts concluded that overall their diversity is not well known. Thorough knowledge of their taxonomy is essential before we can elucidate distribution patterns (Russell and Smith, 1993). Also, it would then be possible to detect new colonizers. The present review considers the terrestrial algae of

Maritime and Continental Antarctic regions, as recognised by Smith (1984b). Latitudinal range is from the northernmost islands of Maritime Antarctica (Bouvet • ya, 54^0 25′ S) to mountains in the Queen Maud Range (86^0 30′ S).

The first detailed descriptions of Antarctic algae were provided by West and West (1911) and Fritsch (1912a, b) following collections made by the expeditions of Shackleton and Scott during 1901-1911. Their brief descriptions and illustrations, based on preserved material, have since been used extensively as a basis for identifications by Antarctic researchers. These and other taxonomic studies, and knowledge of the distribution of algae up to the early 1960s, have been reviewed by Hirano (1965) and Koob (1967).

An extensive species list is presented by Prescott (1979) who included a bibliography up to 1977 of Antarctic and Subantarctic algae, together with a checklist of freshwater taxa. There is no up-to-date checklist which covers literature after 1977. A summary of selected studies on Antarctic non-marine algae is shown in Table 1.1. These studies are those which can be regarded as significant for a locality. The number of recorded species is often increased as more habitats are effectively sampled. Also, there is some evidence that the richness of the flora decreases with increasing latitude. Eleven genera have been cultured from mineral soils and visible mats in small ponds collected in the Queen Maud Range (Claridge *et al.,* 1971; Cameron, 1972a) and Pensacola Mountains (Parker *et al.,* 1982). In contrast, nunataks closer to the coast support a richer flora, e.g. 19 genera from Drønning Maud Land (Engelskjøn, 1986; Ryan *et al.,* 1989) and 14 genera from Edward VII Peninsula (Broady, 1989a). This increases even further at coastal locations such as Vestfold Hills where 52 genera were recorded by Broady (1986). In many Antarctic localities, there is opportunity for more detailed work, which will almost certainly result in more species being found.

As well as insufficient exploration of some areas, there are a number of other factors which account for the underestimation of numbers of chlorophyte and xanthophycean species in Antarctica. There is no single flora on Antarctic chlorophyte and xanthophycean algae, and workers have based their identifications on floras of other parts of the world, and therefore may be failing to recognize new species. Much

previously published work is of little help in creating a reliable taxonomy unless identifications are supported by thorough descriptions, illustrations and herbarium specimens. This has also been stressed for studies of diversity and distribution of diatoms (Mann and Droop, 1996; Jones, 1996). There is therefore a need for greatly improved documentation of Antarctic chlorophyte and xanthophycean algae.

Table 1.1. A summary of selected post-1977 floristic studies on non-marine Antarctic algae.

Literature	Locality	[a]Number of species recorded from Antarctica					
		Cyano.	Dino.	Xantho.	Hetero.	Chloro.	Crypto.
Seaburg *et al.*, 1979	Southern Victoria Land	33	0	3	37	28	1
Hirano, 1979	Syowa Station	42	0	0	0	16	0
Broady, 1979a	Signy Island, South Orkney Island	49	1	10	10	39	0
Broady, 1986	Vestfold Hills	34	0	8	8	36	0
Broady, 1987a	Northern Victoria Land	22	0	3	13	19	0
Broady, 1989a	Edward VII Peninsula	19	0	0	0	11	0
Pankow *et al.*, 1991	Schirmacher Oasis	100	1	2	58	56	0
Ohtani *et al.*, 1991	Syowa Station	11	0	0	4	8	0
Luscinska and Kyc, 1993	King George Island	25	0	2	125	31	0
Ling and Seppelt, 1998	Windmill Islands Region	50	1	9	12	70	1

[a] Cyano., Cyanophyta (= Cyanobacteria); Dino., Dinophyta; Xantho., Xanthophyceae; Hetero., Other Heterokontophyta, including Chrysophyceae and Bacillariophyceae; Chloro., Chlorophyta; Crypto., Cryptophyta and 0, none were found. Classification based on Hoek *et al.* (1995). Literature prior to 1977 is included in the checklist by Prescott (1979).

We have insufficient baseline knowledge of the chlorophyte and xanthophycean algae from Antarctica and of their seasonal changes in abundance and species diversity. The latter is vital for assessing endemism and cosmopolitanism. Terrestrial algae are

generally regarded as cosmopolitan (Archibald, 1990; Starks *et al.*, 1981). Many have been recorded from Antarctica but there is limited evidence for Antarctic endemism. However, the adoption of a more restricted species concept may well reveal a greater number of endemic species. Until sufficient studies have been made using a more reliable taxonomy it will not be clear how many species are endemic and how many are truly cosmopolitan.

Detailed floristic accounts are available for only South Orkney Islands (Broady, 1979a), Schirmacher Oasis (Pankow *et al.*, 1987, 1991), ice-free areas of Lützow-Holm Bay (e.g. Akiyama,1967; Ohtani, 1986), Vestfold Hills (e.g. Broady, 1986) and southern Victoria Land (e.g. Seaburg *et al.*, 1979; Friedmann *et al.*, 1988). However, there are inadequacies even in these studies. The algal flora of Antarctica remains poorly known.

Up to 1998, 441 species of chlorophytes and 63 of xanthophyceans have been recorded from terrestrial and freshwater habitats from Antarctica (Appendix 1, Table 1.2). In comparison with chlorophyte algae, little attention has been given to xanthophyceans.

Table 1.2. Total number of chlorophyte and xanthophycean species recorded from freshwater and terrestrial habitats in Antarctica.

Division / [a]Class	Total number of species
Chlorophyta	441
Chlamydophyceae	86
Chlorophyceae	223
Ulvophyceae	32
Charophyceae	25
Zygnemaphyceae	75
Heterokontophyta	
Xanthophyceae	63

[a] According to Ettl and Gärtner (1995)

A survey of the taxonomic literature on terrestrial Antarctic chlorophyte and xanthophycean algae is provided in the following sections in order to obtain an overall picture of the present state of our knowledge. The techniques which have been

employed for description of these are described first. Then follows a review of their diversity and distribution patterns and an outline of the objectives of the present study.

1.2 An assessment of knowledge of the diversity of yellow-green and green Antarctic terrestrial and non-marine aquatic algae

1.2.1 Techniques utilised

All general reviews of terrestrial algae worldwide (e.g. Hoffman, 1989; Nienow, 1996) have stressed the necessity of using a range of techniques for identifying all species of algae. In no region of Antarctica have terrestrial algae from all habitats been studied using a range of techniques for identification of all species. Broady (1979a) discussed the success of combining direct microscopic examination and cultures, i.e. mixed species enrichment cultures and unialgal cultures on defined media, for identification of the maximum number of species.

Broady (1996) recommended the greater use of cultures and molecular approaches to increase knowledge of the diversity of Antarctic algae. There are only two examples of molecular genetics, namely DNA/DNA hybridization and 18S ribosomal RNA gene-sequencing, applied to Antarctic cyanobacteria (Nienow and Friedmann, 1993) and the chlorophyte *Bracteacoccus* (Lewis, 1997) respectively. The latter confirmed that all Antarctic *Bracteacoccus* strains are closely related to strains from USA, Japan and Europe.

A large number of species has been identified based on direct microscopic examination of field materials (Table 1.3). For example, Pankow *et al.* (1991) recorded 217 species from Schirmacher Oasis and Broady (1987a) listed 59 taxa from northern Victoria Land including aquatic freshwater algae. The contact slide method is a convenient method for direct counting of soil microbes and for identification of soil algae *in situ* (Pipe and Cullimore, 1980). Ohtani *et al* (1991) used this method to investigate *in situ* distribution of soil algae in relation to available water. 23 taxa were recognised from the slides.

164 taxa (Tables 1.3, 1.4) have been identified using culture techniques. Without cultures, life-cycle stages can be mistakenly described as independent species. For instance, the careful examination, in culture, of what have been regarded as resting stages of *Chlamydomonas nivalis* (Bau.) Wille, recorded from Windmill Islands (Llano, 1965), shows that these are in fact a new species of *Chloromonas* (Ling and Seppelt, 1993). Antarctic snow algae collections also commonly record *Scotiella* spp. (e.g. Corte, 1966; Kol, 1971; Akiyama, 1979) but culture studies have shown these to be zygotes of *Chloromonas* spp. (Hoham *et al.,* 1983).

Table 1.3. Total number of species of chlorophyte and xanthophycean algae recovered by each of two methods of sample examination.

Taxa	Total number of species	[a]Number of species recovered by each technique	
		Direct microscopic examination	[b]Culture
Chlorophyta	441	277	164
Xanthophyceae	63	26	37

[a] for literature see Table 1.4

[b]mixed species enrichment culture and/or isolation as unialgal cultures.

Limited ultrastructural studies have been made on chlorophytes (Broady, 1987b; Broady and Ingerfeld, 1993; Ling, 1996) and xanthophyceans (Broady *et al.,* 1997; O`Kelly, 1989). Broady (1987b) described *Pseudococcomyxa simplex* (Mainx) Fott using light and electron microscopy and corrected previous misidentifications of this alga as *Monodus subterraneus* J.B. Petersen (Broady, 1979a). Also, *Hormidiospora verrucosa* was mistakenly identified as *Heterothrix antarctica* (Broady, 1976) based on light microscopic (LM) studies (P. Broady, pers. comm.). Transmission electron microscopy (TEM) has shown starch granules in the chloroplasts and the characteristic ultrastructure of a chlorophyte. Therefore, electron microscopy can be essential for confident identification of certain chlorophyte and xanthophycean algae. In addition to

Table 1.4. Different techniques used in taxonomic studies of Antarctic terrestrial and freshwater algae.

Literature	Direct microscopic examination	Mixed species enrichment culture	Isolation as unialgal culture
Akiyama, 1967	-	+	-
[b]Akiyama, 1979	+	+	-
Broady, 1979a, b	+	+	+
Broady, 1981 a, b, c, 1982c, 1986	+	+	+
Broady, 1984b	+	-	+
[a]Broady, 1987b	-	-	+
[a]Broady and Ingerfeld, 1993	-	-	+
Broady *et al.*, 1987	+	-	+
[a]Broady *et al.*, 1997	-	-	+
Cameron, 1972a	-	+	_
Cameron and Benoit, 1970	-	+	-
Claridge *et al.*, 1971	-	+	-
Darling *et al.*, 1987	-	-	+
Holm-Hansen, 1964	+	+	+
Kol, 1968, 1973	+	+	+
Kol and Flint, 1968	+	-	+
[ac]Ling, 1996	+	-	+
[a]O'Kelly, 1989	-	-	+
Parker *et al.*, 1982	-	+	-
Seaburg *et al.*, 1979	+	+	+

[a] TEM used for ultrastructural studies;

[b] SEM used on *Scotiella* and *Cryocystis*;

[c] SEM used on *Chloromonas polyptera* and *Desmotetra* spp;

+, technique used; -, technique not used.

ultrastructure by TEM observation, two unequal flagella are one of important taxonomic characters of xanthophycean algae in order to distinguish them from chlorophytes species.

The large majority of algae have been described only by direct microscopic examination which leads to low confidence in many identifications (Tables 1.3, 1.5). Whereas desmids can often be reliably identified by this approach because of their distinct morphology, chlorophyte and xanthophycean algae can be identified with high confidence often only after their isolation as unialgal cultures. Unicellular taxa in particular can need prolonged study in culture of their morphology and reproduction before they can be identified (Broady, 1979a, 1996). Much descriptive work on isolates in unialgal culture must be performed before the terrestrial flora is fully known.

Table.1.5. Degree of confidence in identification of chlorophyte and xanthophycean species based on direct microscopic examination and culture techniques.

Taxa	Confidence in identification		
	[a]Low	[b]Medium	[c]High
Chlorophyta	182	33	226
Xanthophyceae	23	3	37

[a]taxa are incompletely characterised based on direct microscopic examination or described as "unidentified" taxon.

[b]algae are tentatively assigned to a taxon.

[c]species are described/listed based on mixed species enrichment cultures and/or unialgal cultures. Out of the 226 chlorophyte species, eight have been studied using electron microscopy. This category includes 55 species from the Desmidiaceae which have been identified with confidence based on direct microscopic examination because of their distinct morphology.

1.2.2 Xanthophyceae

Xanthophyceae have not been studied as extensively as most other algae even though species are fairly cosmopolitan (Tarapchak, 1972). Close to 70% of species are coccoid unicells found in still waters and soils (Ott, 1982). Taxonomy of the class has been monographed by Pascher (1939) and this has been revised by Ettl (1978). Some nomenclatural problems have been dealt with by Silva (1979). In this section, knowledge of the taxonomy of xanthophycean algae from Antarctica is assessed.

Sixty-three species have been recorded from Antarctica (Appendix 1; Tables 1.3, 1.5). Four genera, *Botrydiopsis* Borzi, *Monodus* Chodat , *Xanthonema* Silva (= *Heterothrix*) and *Heterococcus* Chodat are commonly recorded (Broady, 1996). Thirty taxa are described by LM using cultures and only four (three taxa from *Xanthonema* and one from *Heterococcus*) described by TEM.

Four taxa are considered as "doubtful" because of unreliable identification. They are recorded as "unidentified genus of the family Pleurochloridaceae" (Broady, 1979a) and tentatively assigned to the taxa, *Chlorellidium* (Broady, 1987a; Broady and Smith, 1994), *Ellipsoidion perminimum* (Broady, 1979a) and *Heterothrix montana* (Broady, 1984a). Twenty-six species (Table1.3) are unreliably identified by direct microscopic observation (e.g., Fritsch 1912b; Corte, 1962; Broady, 1987a; Pankow *et al.,* 1991).

Kol and Flint (1968) described a new variety, *Ellipsoidion perminimum* var. *cryophila* Kol and a new species *Chloridella glacialis* Kol, from green ice of Balleny Island. Ettl (1978) regarded both taxa as probable *Chlorella* spp. because of a lack of pigmentation data. The algae have to be re-examined using pigment analysis, TEM or molecular genetic studies for their confident placement in either Chlorophyta or Heterokontophyta.

There is a lack of detailed descriptions and adequate illustrations for Antarctic xanthophycean algae that often make their identity uncertain. For example, *Nephrodiella semilunaris* Pa. is recorded by Fumanti *et al.* (1993) but without descriptions or illustrations. Starmach (1995) describes *Chlorocloster pyreniger* Pa. var. *minor* and *C. terrestris* Pa., but he does not mention reproduction in either species.

Records (e.g., Starmach, 1995) of *Pleurochloris commutata* Pa. and *P. polychloris* Pa. need confirmation as zoospores were not observed in either species. Neither the record of *Bumilleriopsis brevis* (Cameron and Benoit, 1970) nor *Bumilleriopsis* sp. (Flint and Stout, 1960), are supported by descriptions and illustrations. A record of *Bumilleria* from southern Victoria Land was accompanied by a brief, unconvincing description and there was no illustration (Holm-Hansen, 1964) This could easily be a species of *Xanthonema.*

Examination of pigments or ultrastructure are needed for reidentification of some inadequately described specimens. For instance, following re-examination of an alga identified as *Gloeobotrys terrestris* Reisigl (Broady, 1979a), it is now thought to closely resemble the chlorophyte *Coccomyxa gloeobotrydiformis* Reisigl (Broady, pers. comm.). Very similar cultured specimens from Vestfold Hills (Broady, 1982c) contain chlorophyll *b* and starch.

Broady *et al.* (1997) examined ten poorly known Antarctic strains of *Xanthonema* by LM and TEM. This detailed investigation identified *X. sessile* (Vinatzer) Ettl and Gärtner and *X. hormidioides* (Visher) Silva as new records for Antarctica. This is a difficult genus where distinguishing characteristics of species are often slight (cell dimensions, zoospore characteristics) and in need of revision. There are no records of pyrenoids being detected in *Xanthonema* using LM. Some Antarctic strains have stalked pyrenoids which are only visible using TEM (Broady *et al.,* 1997).

Heterococcus contains at least 52 species (Ettl and Gärtner, 1995) which can be distinguished only after careful and prolonged examination of unialgal cultures (Lokhorst, 1992). Darling *et al.* (1987) performed such a study in which they established *H. endolithicus* as a new species from Antarctica. Broady (pers. comm.) observed variability in morphology of an isolate of *Heterococcus* when cultured at different salinities. Such morphological plasticity stresses the need for comparison of cultured strains using standard conditions of culture. Several records of *Tribonema* are from just field material, e.g. *T. bombycinum* (Ag.) Derbés and Solier (Fritsch, 1912a, b; Corte, 1962), *T. elegans* Pa. (Broady, 1982b), *T. glacialis* Kütz. (Lavrenko, 1967) or based on preserved material, e.g. *T. ulotrichoides* Pa. (Luscinska and Kyc, 1993) and are

very unreliable. Therefore, a comparative, traditional and molecular genetical study would be valuable for Antarctic isolates, including comparisons with isolates from elsewhere.

Coccoid genera, particularly *Botrydiopsis, Botryochloris* Pascher and *Chlorellidium* Vischer and Pascher, are poorly known from Antarctica and emphasis is given to these in the present study. Out of 13 species of *Botrydiopsis,* five have been reliably described (Ettl and Gärtner, 1995). Of the latter, Antarctic specimens of *B. arhiza* Borzi (Akiyama, 1968; Broady, 1986) and *B. intercedens* Pa. (Broady, 1986) have been briefly described while *B. constricta* Broady (Broady, 1976, 1979a, b) has been more adequately described. Also, *Botrydiopsis* has been recorded from different locations in Antarctica without assigning specimens to any particular species, e.g. from Edmonson Point (Broady, 1987a); Marie Byrd Land (Broady, 1989a); Scott Base (Broady and Smith, 1994); Cape Bird (Broady, 1989c); Signy Island (Broady, 1979b); Windmill Islands region (Ling and Seppelt, 1998).

A single species of *Botryochloris, B. minima* Pascher has been described from Antarctica at Thala Hills Oasis, Enderby Land (Starmach, 1995). Description of the vegetative stage fits the genus. However, zoospores were not observed. Their production is an important diagnostic character for the genus. Therefore, species, and even generic, designation of Antarctic specimens requires confirmation.

Chlorellidium contains two species which are found in soil, *C. tetrabotrys* Vischer and Pascher and *C. astigmatum* Schwarz, (Ettl and Gärtner, 1995). All published records of this genus from Antarctica, i.e. as *C. tetrabotrys* and *Chlorellidium* sp. (Broady, 1986, 1987a; Broady and Smith, 1994) are unaccompanied by descriptions and illustrations.

1.2.3 Chlorophyta

Without the use of unialgal cultures full details of the life-cycle remain unknown and necessary information on cytology of vegetative cells, e.g. chloroplast structure, can be difficult to obtain. Therefore, many records which have not used cultures are doubtful.

Of the 441 chlorophyte species known from Antarctica, 226 are based on LM studies of cultures and seven use TEM (Table 1.5). The remaining species were recorded following only direct microscopic examination of field specimens (e.g., Broady, 1987a; Fritsch, 1912b; Pankow *et al.,* 1991). Many records from Antarctic regions require confirmation, for example, *Palmellopsis gelatinosa* Korshikov (Parker *et al.,* 1972); *P. texensis* (Groover and Bold) Ettl and Gärtner (Broady, 1986); *Borodinella polytetras* Miller (Starmach, 1995), and *Dictyochloropsis* sp. (Broady, 1989a).

Identifications of unicells are often left at generic level even when unialgal cultures are established, for example, frequent records of *Bracteacoccus* Tereg, *Chlorococcum* Menighini, and *Tetracystis* Brown and Bold (e.g., Holm-Hansen, 1964; Seaburg *et al.*, 1979; Akiyama *et al.*, 1986a; Broady, 1986). Records often include the category "unidentified unicells" (e.g., Broady 1987a, 1989a; Ohtani, 1986; Ohtani *et al.,* 1991; Ryan *et al.,* 1989) or "unidentified filamentous alga" (e.g., Akiyama *et al.*,1991; Ohtani, 1986; Luscinska and Kyc, 1993).

Chlorococcum is a particularly problematical genus. Ettl and Gärtner (1995) compile descriptions and illustrations of 22 species of *Chlorococcum* from terrestrial habitats. Four species have been recorded from Antarctica, *C. humicolum* (Näg.) Rabenh., *C. infusionum* (Schrank) Menegh., *C. ellipsoideum* Deason and Bold (as *Hypnomonas* ellipsoidea Korsch.) and *C. lobatum* Fritsch and John (as *H. lobata* Korshikov). Most records, 16 in total, go no further than a generic identification and do not provide sufficient detail for worthwhile comparison with species in Ettl and Gärtner (1995). Some of these cannot even be confidently assigned to the genus *Chlorococcum.*

No good descriptions are attached to Antarctic records of *Neospongiococcum* sp. (Broady, 1986, 1987a; Broady and Smith, 1994) and *Spongiococcum* cf. *multinucleatum* (Luscinska and Kyc, 1993). The latter record provides no written description and a very poor illustration made from preserved field specimens. Likewise, there are nine records of *Tetracystis* sp. which provide little or no description. Only one record (Seaburg *et al.,* 1979) compares specimens with a species, namely *T. cf. intermedia.* However, neither the description nor illustration indicates a particularly close resemblance to that species as described by Ettl and Gärtner (1995).

There have been numerous records of named and unnamed species of *Chlorella* (see Appendix 1). Among them, only *C. emersonii* Shih. and Krauss (Broady, 1984b; Broady *et al.,* 1987), *C. saccharophila* (Gern.) Fott and Nováková (Broady, 1984b) and *C. protothecoides* Krüger (Broady, 1984b) are accompanied by reasonable descriptions and illustrations based on cultures. Other records are either simply names in lists or provide inadequate descriptions and illustrations.

Bracteacoccus minor var. *glacialis* Fott is recorded by Pankow *et al.* (1991) based on unreliable direct microscopic observation. *Protoderma brownii* Fritsch, a poorly known alga, recorded by Fritsch (1912b) and Wille (1924) is based on preserved material and needs to be cultured. All Antarctic records of *Binuclearia, B. tatrana* Wittr. in Smith and *B. tectorum* (Küt.) Beger in Wichman are based on direct microscopic examination of field specimens. It is important to culture these algae and examine their morphological variability and reproduction.

The genus *Trebouxia* is not well-characterised in Antarctica. Two Antarctic strains, isolated in culture (Broady, 1979a; Parker *et al.,* 1972), have been seen as free-living cells but species of this genus are usually photobionts in lichens. Only one study has identified species of lichen photobionts (Aoki *et al.,* 1998), these being *T. incrustata* Ahmadjian and Gärtner and *T. cf. impressa* Ahmadjian, but most do not (e.g. Schofield and Ahmadjian, 1972; Meyer *et al.,* 1988). This genus must be widespread in Antarctica as a lichen photobiont and needs detailed study and comparison with species known from elsewhere.

There are numerous records of *Stichococcus* from Antarctica based on cultures and field specimens. *S. bacillaris* Nägeli and unidentified species are most frequently recorded. This genus is in need of close examination and possibly revision. It is quite likely that this is a "form genus" grouping together morphologically similar but genetically distinct entities. As the chloroplast is often pale and starch is difficult to detect, it is possible that some are xanthophyceans. Therefore, detailed examination is needed to check pigments and chloroplast membranes.

It is clear that the identities of many Antarctic chlorophytes remain uncertain because of i) absence of detailed descriptions and adequate illustrations and ii) usually a reliance on field specimens rather than cultures. Antarctic species need to be reassessed by careful comparison of cultured strains obtained from diverse and widespread localities and habitats.

1.3 Distribution patterns of yellow-green and green algae

1.3.1 Distribution between geographical regions

Koob (1967) concluded that the distribution patterns of Antarctic algae are unclear because of lack of information. He emphasized the need for further extensive collections before patterns would become apparent. However, almost 30 years later, this is still largely true due to different intensities of study at different locations and application of different techniques (Broady, 1996).

It has been suggested that the species diversity of terrestrial Antarctic algae is low because of a combination of isolation and environmental severity (Wynn-Williams, 1996). The Southern Ocean acts as a geographical and climatic barrier between the Antarctic continent and more northerly land masses. Potential colonists must arrive via the aerobiota, animal vectors, human beings or wind-borne sea-spray and derived aerosols.

There is little evidence of Antarctic endemism, e.g. *Hemichloris antarctica* Tschermak-Woess and Friedmann has been found only in southern Victoria Land (Tschermak-Woess and Friedmann, 1984). Also, *Signiosphaera multinucleata* Broady (1977a), a genus and species newly described from Signy Island is unknown elsewhere. It has been absorbed into *Pseudodictyochloris* (Ettl and Gärtner, 1995) but has been retained as a distinct species.

Schemes of phytogeographic subdivision of Antarctica and adjacent ocean areas have been reviewed by Pickard and Seppelt (1984) and Smith (1984b). Terrestrial algae form visible surface crusts and mats in favourable habitats and where external conditions are

unfavourable they occupy protected cryptic habitats. Algae are more prominent in the vegetation of the cold, arid Coastal and Slope Provinces of Continental Antarctica. In contrast, bryophytes and lichens dominate the vegetation in the milder, moister Maritime Antarctica (Smith, 1984b). Water availability assumes great importance in determining algal distribution in the ice-free localities in Continental Antarctica.

The distribution of species between the classes of algae is similar in Maritime Antarctica to that found in Continental Antarctica (Tables 1.2, 1.6). In both regions, there is highest representation of Chlorophyceae followed by Chlamydophyceae. Xanthophyceae is poorly represented compared with Chlorophyceae, Chlamydophyceae and Zygnemaphyceae. Fewest species are from Ulvophyceae and Charophyceae. Algal diversity gradually decreases with increasing latitude within Antarctica (Broady, 1996).

1.3.2 Distribution between localities within the Ross Sea regions

The Ross Sea regions of Antarctica contain diverse landscapes with ice-free coastal localities, offshore islands, inland nunataks and mountain ranges and associated valley systems. Microbial diversity is low with a small number of species occurring under a broad range of environmental conditions throughout the region (Vincent and James, 1996). A lack of examination of all different habitats is evident, despite the several studies performed (e.g. Seaburg *et al.,* 1979; Friedmann *et al.,* 1988; Nienow and Friedmann, 1993). For chlorophyte and xanthophycean algae, our knowledge of species distributions and factors which govern these are still fragmentary. Their diversity is not fully known because of inadequate techniques and collections (e.g. Holm-Hansen, 1964; Broady, 1989a, c). Until recently, 46 chlorophyte and seven xanthophycean genera have been recorded from the Ross Sea region (Table. 1.7).

There is no evidence to date of endemism in the Ross Sea region. However, there is a need to apply molecular techniques to compare biodiversity and genetic characteristics with assemblages elsewhere in Antarctica and with comparable communities in the north polar zone. Vincent and James (1996) proposed hypotheses to help guide future research on the microbial diversity of the Ross Sea region. These derive from the

Table 1.6. Numbers of species[a] of chlorophyte and xanthophycean orders[b] found in Maritime and Continental Antarctica.

Taxa	Number of species	
	Continental	Maritime
Chlamydophyceae		
Chlamydomonadales	7	35
Volvocales	-	11
Tetrasporales	2	4
Chlorococcales	3	19
Chlorophyceae		
Chlorellales	26	132
Gloeotilales	6	15
Prasiolales	2	14
Chaetophorales	2	35
Oedogoniales	-	1
Ulvophyceae		
Ulotrichales	2	22
Pleurastrales	2	3
Charophyceae		
Klebsormidiales	6	19
Zygnemaphyceae		
Zygnematales	9	64
Xanthophyceae		
Mischococcales	8	24
Tribonematales	5	25
Vaucheriales	-	1

[a] for literature see Apendix 1

[b] classification according to Ettl and Gärtner (1995)

-, absence of species may have been due to the small number of samples examined or less intensive analysis of the samples.

Table 1.7. Numbers of recorded species of chlorophyte and xanthophycean algae from the Ross Sea region.

[a]Taxa	Species identified	Records as cf. species	Records as genus only
Chlamydophyceae			
Chlamydomonas	6	3	1
Chloromonas	1		
Furcilia	1		
Brachiomonas		1	
Polytomella			1
Thorakomonas	1		
Chlorococcum	2		1
Neospongiococcum			1
Spongiococcum		1	
Tetracystis	2	1	1
Radiosphaera		1	
Actinochloris	1		
Chlorophyceae			
Dictyochloropsis		1	
Sphaerocystis	1		
Kentrosphaera		1	1
Chlorella	6		1
Elliptochloris	2		
Pseudococcomyxa	1		
Scotiellopsis	1		
Golenkinia	1		
Coccomyxa	3		
Gloeocystis	1	1	
Palmogloea	1		
Oocystis	1		
Westella			1
Bracteacoccus		1	1
Neochloris	1		
Chlorosarcina	1		
Chlorosarcinopsis			1
Planophila		1	

cont.

Table 1.7. (cont.)

[a]Taxa	Species identified	Records as cf. species	Records as genus only
Binuclearia	1		1
Geminella	1		
Microspora	1		
Prasiococcus	1		
Prasiola	4		
Coccobotrys		1	
Desmococcus	1	1	
Protococcus	2		
Oligochaetophora	1		
Ulvophyceae			
Ulothrix	2		1
Urospora	1		1
Trebouxia			1
Charophyceae			
Klebsormidium	1		
Raphidonema	1		1
Stichococcus	4		1
Zygnemaphyceae			
Actinotaenium	1		
Xanthophyceae			
Monodus	1		
Bumilleriopsis			1
Botrydiopsis	1		1
Chlorellidium		1	
Bumilleria			1
Xanthonema	3		
Heterococcus	1		1

[a]Classification based on Ettl and Gärtner (1995) and Komárek and Fott (1983).

conclusion that environmental extremes plus biogeographical isolation control the biodiversity of communities, and that biological interactions (competition, grazing, predation, parasitism) are weak and play a minor role by comparison with temperate latitude ecosystems.

The following sections describe distribution patterns within the Ross Sea regions. The studies are presented in geographical progression from north to south.

A floristic survey of algae at four locations in northern Victoria Land, i.e. Inexpressible Island, Edmonson Point, Cape Washington and the vicinity of the "Gondwana Station" in Gerlache Inlet, has been made by Broady (1987a). 19 chlorophyte and three xanthophycean taxa have been recorded from these localities based on field microscopic observation. The category "green unicells", which occurred in over 30% of all samples, probably comprises a variety of species resolvable only in culture. Thorough floristic surveys using culture techniques for taxonomic purposes have yet to be performed in northern Victoria Land. The flora appear to contain algae which have been observed previously in southern Victoria Land and on Ross Island (West and West, 1911, Fritsch, 1912b; Seaburg *et al.,* 1979; Broady, 1982a). Also two studies from further north, Cape Adare (Fritsch, 1917) and Cape Hallett (Rudolph, 1963) suggest that the flora there is similar, although those investigations were superficial.

Southern Victoria Land contains the largest ice-free area on the continent, covering about 5600km^2 and opening at its eastern extremity into McMurdo Sound and the Ross Sea (Seaburg *et al.*, 1979). Clark (1965) has called such dry valleys "oases" as they possess a less severe climate than the surrounding snow and ice-covered regions. The terrestrial biota is extremely scattered, primarily due to the lack of available liquid water (Cameron, 1972a; Schofield and Ahmadjian, 1972; Parker *et al.*, 1977). Friedmann and Ocampo (1976) have shown that species of endolithic and other rock-associated algae exist within these cold deserts much as they occur in hot deserts of the world.

Holm-Hansen (1964) made collections of algae from Ross Island and southern Victoria Land in order to culture algae for physiological studies. The habitats from which algal samples were obtained included a large fresh-water lake (Lake Vanda in the Wright Dry

Valley), shallow fresh-water ponds, moist sand and rocks, dry sand, and dry rocks. From these samples, 19 species were obtained in unialgal culture. However, only brief descriptions of isolates, without illustrations, were provided. Hence, there can be only limited confidence in the identification.

A floristic and ecological study was conducted on the non-marine algae at locations in Taylor Valley, southern Victoria Land (Broady, 1982b). This included examination in some detail of algae in a freshwater stream formed from meltwaters of the Canada Glacier. Over 37 species, predominantly cyanobacteria, were recorded. A distinct zonation formed by *Binuclearia tectorum, Prasiola calophylla* and *Tribonema elegans* was noted along the length of the stream. There is still considerable confusion regarding the circumscription of species of *Prasiola* (Broady, 1989b). The occurrence of transitional stages between species of *Prasiola* is well known. Broady (1989b) provides detailed descriptions and illustrations of specimens identified as *P. calophylla.* However, he discusses difficulties preventing entire confidence in this identification. Developmental stages of other species, including *P. crispa* (Lightf.) Menegh., resemble the typical ribbons of *P. calophylla.* This problem can be solved only by careful comparison of field and cultured specimens in conjuction with molecular approaches.

Seaburg *et al.* (1979) collected samples from glacial meltstreams and moist and dry soils of southern Victoria Land, but did not record distribution patterns. 28 chlorophytes were recorded from this region, but there were three records of xanthophyceans. The presence of suitable nutrient concentrations for algal growth in the glacial meltwater at its source is shown by rich algal growths in the upper reaches of meltwater streams and by the occurrence of algae, e.g.*Chlamydomonas subcaudata* Wille, and *Tetracystis* sp., in cryoconite holes in the surface of the Canada Glacier (Wharton *et al.,* 1981).

Cryptoendolithic algal communities of the Ross Desert in the McMurdo Dry Valleys comprise two eukaryotic and three cyanobacterial communities (Friedman *et al.,* 1988). The moisture content of the rock substrata affects pH, formation of a primary iron stain and the distribution of microbial communities. The phycobionts of the cryptoendolithic

lichens belong to the genera *Trebouxia* Pumaly and *Pseudotrebouxia* Archibald but several strains are not yet identified.

The steam-warmed lithosols close to the summit of Mt. Erebus, Ross Island provide a habitat of greatly restricted area and unusual environmental conditions. The acid reaction of the soils, the constant supply of moisture by condensation of steam and the regular supply of geothermal heat produce conditions which contrast markedly with those in most continental Antarctic soils (Broady, 1984b). Heating of soils around fumaroles causes zonation of algal growth. The algal flora includes seven taxa (five chlorophytes and two cyanobacteria) not recorded previously from Antarctica. The high proportion of *Chlorella* taxa probably reflects selection by the habitat of algae with an acidophilic response from those in the propagule input. An acidophilic alga which is absent is *Cyanidium caldarium* (Tild.) Geitl. This alga is very sensitive to drying (Smith and Brock, 1973) and this would reduce the possibility of its air-borne dispersal to such an isolated locality. The absence of species from the Xanthophyceae and of diatoms could reflect the intolerance of locally available propagules to the acidic soils. They are frequently encountered in other alkaline to neutral soils of southern Victoria Land.

Similar fumarolic soils to those on Mt. Erebus are found on Mt. Melbourne, northern Victoria Land and the vegetation there also has unique features (Broady *et al.*, 1987). There were no xanthophyceans in the assemblage of 17 species (13 chlorophytes and 4 cyanobacteria). Of the 19 species recorded on the two volcanoes, seven (4 chlorophytes and 3 cyanobacteria) were common to both, four (2 chlorophytes and 2 cyanobacteria) were restricted to Mt. Melbourne and eight (7 chlorophytes and 1 cyanobacteria) were restricted to Mt. Erebus.

Fumarolic activity has also been reported in Marie Byrd Land (Le Masurier and Rex, 1982) but the possibility of vegetation growth has not been investigated. A comparison of biologically investigated fumarolic regions (Cameron and Benoit, 1970; Longton and Holdgate, 1979; Engelskjøn, 1981; Broady, 1984b; Broady *et al.* 1987) in Antarctica reveals both similarities and differences in the nature of their biota. It suggests that airborne dispersal has the potential to distribute a wide range of microscopic propagules

to many Antarctic sites. Whether individual species retain their viability during dispersal and would be capable of growth when deposited on each area of fumarolic ground requires detailed study of the survival properties and growth requirements of each potential colonist and a more detailed analysis of the environment at each site.

Broady (1989c) compared broadscale patterns in the distribution of macroscopic and selected microscopic vegetation at three large, coastal, ice-free regions in the vicinity of Cape Bird, C. Royds and C. Crozier on Ross Island. The frequency of occurrence and absence of different communities contrasted markedly from region to region. This variation is related to the levels of marine salts in the environment, fertilisation by birds, water availability, substratum type and degree of exposure. Green algal crusts of *P. calophylla, cf. Prasiococcus calcarius* and *Klebsormidium* sp. coated the surfaces of soils, gravels and stones at all three areas but were particularly abundant at Cape Crozier. There was no record of xanthophyceans. This study has described distribution patterns of dominant algae and broad groupings of lichens and mosses using a 0.5 by 0.5 km square grid.

Broady (1989a) surveyed the terrestrial algal communities at Edward VII Peninsula, Marie Byrd Land. All 23 nunataks in the Rockefeller and Alexandra Mountains were visited, including those with and without nutrient enrichment from bird colonies. Over 30 species of algae were recorded with cyanobacteria and chlorophytes dominant as in other regions of Continental Antarctica. A single xanthophycean, *Botrydiopsis sp.,* was occassionally observed on bird-influenced soil. Occassionally bird bones were found supporting green algal crusts dominated by *S. bacillaris, cf. Desmococcus vulgaris* and *Pseudococcomyxa simplex.* Although lichens were the most visually prominent vegetation, free-living algae occurred in the widespread non-aquatic habitats as epilithic, chasmoendolithic and edaphic communities as well as being epiphytic on the sparse moss cushions.

The distribution and taxonomy of chlorophyte and xanthophycean algae, of the Ross Sea regions remains poorly known. The generic identity is sometimes in doubt and the species even more frequently so. More detailed studies at each locality would greatly increase known species, as has been found for lichens and bryophytes in floristically

rich Maritime Antarctica (Russell and Smith,1993). However, superficial analyses of partial collections from previously unvisited localities are unlikely to add significantly to our knowledge (Broady, 1996). Exact recording of sample sites on large-scale maps and in geographic information systems would allow future recovery of algae of particular interest. However, little further progress can be made without the greater utilization of cultures in conjunction with direct microscopic observations.

1.3.3 Habitats of yellow-green and green algae

A recent review on distribution of Antarctic terrestrial algae (Broady, 1996) concluded that thorough investigations have rarely been accomplished on every habitat at each locality in Antarctica. The aim of this section is to describe the habitat range in which chlorophytes and xanthophyceans occur.

Epilithic communities. Epilithic algae grow on the exposed surfaces of rocks as thin, dark brown to black crusts and are very infrequently encountered (Broady, 1981b). Their occurrence depends on plenty of moisture at the rock surface (Broady, 1996).

Green algal crusts have been described at Cape Washington, northern Victoria Land (Broady, 1987a) and Rockefeller Mountains, Marie Byrd Land (Broady, 1989a). At high-salinity sites where irrigation is infrequent, or perhaps moisture is gained from high atmospheric humidity, *P. calcarius* often dominates green crusts, e.g. on Ross Island (Broady, 1989c). Pankow *et al.,* (1991) noted its absence from Schirmacher Oases.

Dark thin crusts of epilithic algae observed often on the rock slopes near Syowa Station area (unpublished data). The colonies seemed to develop along the meltwater stream on the rock slope in summer season.

Ulothrix dominates the community where there is frequent irrigation in saline environments, e.g. along the shoreline at Signy Island (Broady, 1979a) and Mawson Rock (Broady, 1982b) and where sea-spray is swept inland at Cape Bird on Ross Island (Broady, 1989c).

All these records are indicative of interactions between moisture availability, salinity and nutrients in controlling species structure of epilithic communities. However, the relative influences of these factors are unknown.

Chasmoendolithic communities. Algae inhabiting rock fissures and cracks which penetrate inwards from the outer surface are termed "chasmoendoliths or chasmoliths" (Golubic *et al.,* 1981). They are seen as vivid green or blue-green growths when rocks are split along existing cracks (Broady, 1981c).

During a general survey of terrestrial algae in Princess Elizabeth Land and Mac. Robertson Land, chasmoendolithic algae were found to be widespread, especially at the Vestfold Hills (Broady, 1982c). Salt levels in the environment are important in determining the species composition of chasmolithic communities at coastal locations (Broady, 1981c). This factor was of particular importance in affecting the distribution patterns of the two most frequent chlorophytan algae. *P. calcarius* occurred only in salt-affected areas and *cf. Desmococcus* sp in areas not affected in this way (Broady, 1981c). Ten chlorophyte algae were found as chasmoendolith at the Vestfold Hills and Mawson Rock (Broady, 1981c, 1982b). Among them, illustrations and descriptions were presented for the six most frequent algae, *Trochiscia* sp., *Chlorella* sp., *Stichococcus* sp., two species of *cf. Desmococcus* and *P. calcarius.* There were no xanthophyceans.

The low salt environment at inland nunataks on Edward VII Peninsula, Marie Byrd Land (Broady, 1989a), appears ideal for the growth of *cf. Desmococcus vulgaris* and similar species. However, the dominant chasmoendolithic algae at these locations are in contrast to those at upland sites with similarly low salt levels in southern Victoria Land where a unicellular, cryptoendolithic, endemic *Hemichloris antarctica* is often accompanied by coccoid cyanobacteria. This difference could be related to the differing rock types or availability of lower moisture in that high altitude desert.

Chasmoendolithic algae are no doubt far more widespread than preliminary searches indicate (Broady, 1981c). In coastal regions, chasmoendolithic algae appear not to be associated with chasmoendolithic lichens which have been reported from inland

localities (Seppelt and Broady, 1988). These free-living algae are more abundant in coastal locations than further inland.

Cryptoendolithic. Cryptoendolithic algae develop within minute cavities inside the rock matrix. Those of high altitude inland areas of southern Victoria Land are the most thoroughly investigated terrestrial algae in Antarctica. They are reviewed in detail by Nienow and Friedmann (1993). Community dominants are either lichens or *H. antarctica* or various cyanobacteria. Xanthophyceans are not recorded. Factors controlling occurrence are unclear.

Hypolithic communities. Hypolithic or sublithic algae develop on the undersurface of quartz stones and other suitable translucent rock types that are partially buried in soil. Hypolithic communities are widely distributed on the undersurface of quartz stones near Syowa Station area (unpublished data). They have been reported to be common only at Vestfold Hills (Broady, 1981b) and Schirmacher Oasis (Pankow *et al.,* 1991). Scarcity of suitable translucent stones in other regions makes this habitat insignificant, e.g. Ross Island (Broady, 1989c).

Dominant taxa closely resemble those of chasmoendolithic communities. At Vestfold Hills, 23 taxa are recorded as hypolithic. Only six are chlorophytes, and *Prasiococcus calcarius* and *cf. Desmococcus* sp. are abundant (Broady (1981b). No xanthophyceans were recorded. At Schirmacher Oasis, the community comprises solely *Aphanocapsa endolithica* Ercegovic.

Edaphic communities. Edaphic or "soil" algae live on or within the upper layers of soil.

Akiyama (1967) reported 32 species of soil algae from Syowa Station area and recognized four species of xanthophycean algae, such as, *Monodus subterraneus, Botrydiopsis arhiza, Bumilleria exilis,* and *Monocilia viridis.*

At Signy Island (Broady, 1979a), there is a wide diversity of species in mineral, brown earth and biogenic soils. Brown earths contained the most diverse community whereas

less diversity was found in mineral soils. Thirty-nine chlorophyte species and 10 xanthophyceans were recorded from these habitats.

In southern Victoria Land there have been detailed studies of algae in mineral soils (Seaburg *et al.*, 1979). 28 species of chlorophytes and three xanthophyceans, *Monodus coccomyxa* Pascher, *Heterothrix bristoliana* Pascher and *Heterococcus moniliformis* Vischer, have been recorded from this region (Seaburg *et al.*, 1979). Cameron (1972b) detected cyanobacteria, using cultures, in only one soil in Victoria Valley, but chlorophytes and xanthophyceans were not recorded. However, Broady (1996) observed widespread microscopic communities dominated by *Botrydiopsis* in depressions bordering frost polygons.

Ten species have been recorded from soil of Edward VII Peninsula, nine of them chlorophytes. Only a single xanthophycean, an unidentified *Botrydiopsis* sp., was observed (Broady, 1989a). This was infrequent on bird-influenced soil.

Seventeen chlorophytes and four xanthophycean algae have been recorded from soils of the Vestfold Hills (Broady, 1981b). *P. crispa* and *P. calcarius* were abundant. *Botrydiopsis cf. arhiza* , *B. cf. intercedens* and *Heterococcus* spp. occurred on biogenic soil.

The strong selective effect of environmental factors is emphasized by occurrence of distinct communities on heated fumarolic ground at the summits of Mt. Erebus, Ross Island (Broady, 1984b) and Mt. Melbourne (Broady *et al.*, 1997). Xanthophytes and diatoms are absent from these habitats. This is possibly a reflection of the somewhat acidic reaction of the soils (Broady, 1996).

In addition, a well-developed fumarole vegetation has also been reported for the South Sandwich Islands (Longton and Holdgate, 1979; Smith 1984a, 1984b) and Deception Island (Collins, 1969), but no detailed study on chlorophytes and xanthophyceans was made.

Epiphytic communities. Mosses grow only where there is a good supply of moisture and nutrients. Epiphytic algae are always present as microscopic growths and often form visible crusts over bryophyte surfaces.

At Signy Island, Broady (1979a) compared dry moss-cushion fellfield, moist moss turf, and wet bryophyte carpets. Xanthophycean algae, e.g. *Chloridella neglecta* Pascher and *Tribonema vulgare* Pascher, occurred in more than 50 percent of dry moss-cushion fellfield samples. However, chlorophytes were less frequent as epiphytes. *P. simplex* (Broady, 1977b, 1979a) was more frequent in moss turf than moss carpet.

In Continental Antarctica, floristic accounts of epiphytic communities have been provided for Lützow-Holm Bay area (Ohtani, 1986; Ohtani and Kanda, 1987), Vestfold Hills (Broady, 1986) and Schirmacher Oasis (Pankow *et al.,* 1991). Crust-forming communities are generally dominated by cyanobacteria. Where there is salt efflorescence or nutrient supply from birds the cyanobacteria can be replaced by green crusts of chlorophytes, i.e. *Tetracystis* sp., *S. bacillaris, P. calcarius* and unidentified unicells (Broady, 1986).

A single species of desmids, *Actinotaenium cucurbita* (Bréb. in Ralfs) Teiling was frequent in moss colonies in the vicinity of Syowa Station (Ohtani, 1986). However, *Cosmarium clepsydra* Nord. rarely occurred on mosses. Ohtani and Kanda (1987) also recorded several species (19 cyanobacteria, 1 diatom and 1 unicellular green alga) as epiphytes on the moss *Grimmia lawiana* Willis near Syowa Station. There was no record of xanthophyceans.

Cryophilic communities. Snow algae are largely restricted to Maritime Antarctica and the coastal fringe of Continental Antarctica.

At Signy Island, snow algae, chiefly *Chlamydomonas, Raphidonema* and *Ochromonas* species, are common on the surface of snow during summer (Fogg, 1967).

Ohtani (1998) observed *Botrydiopsis* sp. in the green sherbet snow which dominated by *Klebsormidium* sp. at Fildes Peninsula of King George Island.

Ryan *et al.* (1989) found green snow to be inhabited by *Chlorella* sp. *Prasiola crispa* (Lightfoot) Meneghini, *cf. Coccobotrys* Chodat and *Stichococcus* sp. at nunataks in Drønning Maud Land. This is the furthest inland record of cryophilic communities from Antarctica. There was no record of xanthophyceans.

On Ross Island, green snow found at Cape Royds are the furthest south record of snow algae and Cape Crozier, but the absence of snow algae at Cape Bird may be related to the general lack of large snowdrifts, persisting throughout summer, in areas traversed by penguins (Broady, 1989c).

A saccoderm desmid, *Mesotaenium berggrenii* (Wittr.) Lagerheim, and a volvocalean alga, *Chloromonas rubroleosa* Ling and Seppelt, occurred as major components of grey and red snow respectively, at the Windmill Islands (Ling and Seppelt, 1990, 1993). Until recently, 24 algal taxa, 19 from Chlorophyta and one, *Ellipsoidion* sp.? from Xanthophyceae, have been identified from snow and ice samples from Windmill Islands region (Ling, 1996; Ling and Seppelt, 1998). *Desmotetra* Deason spp, *Chlorosarcina* Gerneck sp., *Chloromonas rubroleosa, C. polyptera* Fritsch and *Palmellopsis* sp. Korsch. have been described as new species from this region (Ling, 1996). Green snow is widespread on the islands where wind-borne sea spray and guano from the many penguin colonies ensure a high nutrient content. There is a further distinction within the green snow group, the *Desmotetra* spp. appearing to prefer snow of lower conductivity (6-85 $\mu S\ cm^{-1}$) than snow containing *Palmellopsis* sp. (Ling and Seppelt, 1998).

There have been very few attempts at characterization of the environment in which algae exist. The importance of this is clearly demonstrated by the studies on cryptoendolithic communities (Nienow and Friedmann, 1993) where variations in light, moisture and temperature over the millimetre scale profoundly affect distribution patterns. Measurements of a range of physico-chemical factors at this scale within the living space of algal communities would enable correlation with floristic data. This needs to be achieved in all terrestrial habitats.

The widespread distribution and abundance of chlorophyte and xanthophycean algae in at least some habitats suggests that they are worthy of study.

1.3.4 Gaps in knowledge

Our knowledge of the distribution of chlorophyte and xanthophycean algae in Antarctica is still fragmentary. The main reasons for this are: 1) the lack of floristic studies in many regions of Antarctica, 2) deficiencies in sampling all habitats at each locality investigated and 3) the low level of taxonomic knowledge of these algal groups. Endemic taxa and cosmopolitan forms of low abundance may be overlooked.

Diverse chlorophyte and xanthophycean algae have been identified from Antarctica, however, many studies are superficial and taxononomic details remain unanswered. The existence of many incomplete descriptions and the lack of application of appropriate techniques have made comparisons between studies difficult. Also, a lack of taxonomic consistency, coupled with insufficient collection from some geographical areas, make the estimation of the number of Antarctic chlorophyte and xanthophycean species problematic.

1.4 Aims of the present study

The taxonomy of many Antarctic algae remains poorly investigated. There is a need for an up-to-date check list of all Antarctic records. Without this our knowledge of Antarctic biodiversity will remain poor.

Terrestrial algae are generally regarded as cosmopolitan but it is possible that due to the isolation and harsh environment of Antarctica novel taxa occur there. The present study aims to contribute to the resolution of the following hypothesis: "The terrestrial algal flora of Antarctica does not comprise cosmopolitan species". Attention has been concentrated primarily on the poorly known unicellular xanthophyceans and secondarily on unicellular and filamentous chlorophytes.

The aims of the study are as follows:

- To provide detailed descriptions, using light and electron microscopy, of the vegetative and reproductive characteristics of unknown or previously poorly known

xanthophycean and chlorophyte algae isolated from the Ross Sea regions of Antarctica. Emphasis is given to 17 poorly known genera. These are:

Heterokontophyta, Xanthophyceae

Botrydiopsis, Botryochloris and *Chlorellidium.*

Chlorophyta

Chlamydomonas, Chlorococcum, Neospongiococcum, Radiosphaera, Tetracystis, Macrochloris, Myrmecia, Chlorella, Elliptochloris, Bracteacoccus, Binuclearia, Protoderma, Trebouxia and *Stichococcus.*

- To use isozymes to investigate genetic relationships between isolates of particular morphospecies of xanthophyceans from increasingly separated origins. How similar are isolates from a) the same sample material b) different samples at the same locality c) different localities within Antarctica and d) Antarctica and other regions?

- To compare congruency of classifications of xanthophyceans based on morphological criteria and on isozymes.

- To investigate carotenoid pigments as taxonomic characters within *Botrydiopsis* and *Chlorellidium.* Do different morphotypes of each genus have characteristic pigment signatures?

2. METHODS AND MATERIALS

2.1. Origin of strains

2.1.1. Culture collections

Most strains for this study were derived from the PAB (Paul A. Broady, University of Canterbury, New Zealand) culture collection. This comprises strains obtained from widespread localities in Antarctica and from Christchurch, New Zealand (Table 2.1). In addition to these strains additional ones were obtained from the N.E.R.C. Culture Collection of Algae and Protozoa (CCAP), U.K. and from Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen (SAG), Germany.

2.1.2. Antarctic sample materials

Additional strains were isolated from samples which had been obtained from: a) Edward VII Peninsula, Marie Byrd Land, b) the Cape Geology/Botany Bay ice-free area on the southern shoreline of Granite Harbour and c) Castle Rock, Ross Island. All samples were collected into sterile polythene bags by Dr. Paul A. Broady and stored at -15^{0}C. Ten samples from each of Granite Harbour and Marie Byrd Land, and six from Castle Rock, were used to isolate 55 strains. Their generic identification, habitat and location are given in Table 2.1.

2.2. Growth and isolation of algae

2.2.1. Media and incubation conditions

Bold`s Basal Medium (BBM; Tompkins *et al.*, 1995), was used for all cultures. All media were sterilised by autoclaving at 121^{0}C for 15 minutes (15 p.s.i.). 1% w/v agarised medium was used for slopes in screw-capped polycarbonate tubes on which

Table 2.1. Identification to generic level of strains examined in this study and a summary of the origin of each strain.

Strain number	[a]Location	[b]Habitat	[c]Isolator	Date of isolation	Original strain number and identification
Heterokontophyta, Xanthophyceae					
Botrydiopsis Borzi					
485	CH	US	BRO	1979	
638	EP	US	BRO	1985	
645	CC	US	BRO	1985	
724	LH	UL	OHT	1990	SO 11
829	VV	US	BRO	1991	
836	VV	US	BRO	1991	
837	VV	US	BRO	1991	
864	CHCH	US	BRO	1989	
877	CHCH	US	BRO	1989	
886	KGI	CR	OHT	1991	KG58
894	SI	MS, BM	BRO	1974	[d]CCAP 806/4, *B. constricta*
895	UK	US	PRI	?	CCAP 806/2, *B. arhiza*
896	SW	US	VIS	1940	[d]SAG 806/1, *B. alpina*
897	AU	BV	TSC	1979	SAG 243/80, *B. alpina*
908	CA	US	LIN	1989	Ling
801	SB	CR	BRO	1991	
G 41/6	GH	MS	BEG	1996	
G 12/1	GH	BM	BEG	1996	
G 94/1	CR	MS	BEG	1996	
G 9/8	GH	BM	BEG	1996	
G 19/2	GH	BV	BEG	1996	
G 24/8	GH	HY	BEG	1996	
G 27/1	GH	BV	BEG	1996	
G 31/5	GH	BV	BEG	1996	
G 99/2	CR	MS	BEG	1996	
G 27/2	GH	BV	BEG	1996	
WI /1	CA	MS	LIN	1989	Ling 99
Botryochloris Pascher					
G19/1	GH	BV	BEG	1996	
Chlorellidium Vischer and Pascher in Vischer.					
597	CB	US	BRO	1983	
757	SB	AP	BRO	1991	
758	SB	AP	BRO	1991	
785	SB	[e]US	BRO	1991	
871	CHCH	US	BRO	1989	
898	SW	US	VIS	1935	[d]SAG 811/1, *Ch. tetrabotrys*
G 28/7	GH	MS	BEG	1996	
G 31/6	GH	BV	BEG	1996	
Chlorophyta					
Binuclearia Wittrock					
762	SB	US	BRO	1991	

cont.

Cont. Table 2.1.

Strain number	[a]Location	[b]Habitat	[c]Isolator	Date of isolation
Bracteacoccus Tereg				
G 12/6	GH	BM	BEG	1996
Chlamydomonas Ehrenberg				
751	SB	US	BRO	1991
766	SB	US	BRO	1991
G 99/4	CR	MS	BEG	1996
Chlorella Beijerinck				
635	II	US	BRO	1985
637	EP	US	BRO	1985
639	EP	US	BRO	1985
752	SB	US	BRO	1991
826	VV	US	BRO	1991
828	VV	US	BRO	1991
G 8/6	GH	BV	BEG	1996
G 27/4	GH	BV	BEG	1996
G 9/1	GH	BM	BEG	1996
G 28/3	GH	MS	BEG	1996
G 31/1	GH	BV	BEG	1996
MB 49/6	MB	BS	BEG	1996
Chlorococcum Meneghini				
633	II	US	BRO	1985
765	SB	US	BRO	1991
823	VV	US	BRO	1991
775	SB	CR	BRO	1991
821	VV	US	BRO	1991
[f]*Diplosphaera* Bialosuknia em. Vischer				
JC 41D	SI	AP	MAR	1994
G 95/8	CR	BV	BEG	1996
G 97/2	CR	BV	BEG	1996
G 98/7	CR	BV	BEG	1996
MB 38/1	MB	BV	BEG	1996
Elliptochloris Tschermak-Woess				
ISO 15	SI	AP	MAR	1994
TH 101RR/L	SI	AP	MAR	1994
FB 102RR	SI	AP	MAR	1994
G 19/5	GH	BV	BEG	1996
MB 38/3	MB	BV	BEG	1996
G 41/2	GH	MS	BEG	1996
MB 42/3	MB	HY	BEG	1996
Macrochloris Korshikov				
G 8/7	GH	BV	BEG	1996
G 9/4	GH	BM	BEG	1996
809	VV	US	BRO	1991

cont.

cont. Table 2.1

Strain number	[a]Location	[b]Habitat	[c]Isolator	Date of isolation
Myrmecia Printz				
FB 45D	SI	AP	MAR	1994
Neospongiococcum Deason				
761	SB	US	BRO	1991
Protoderma Kützing				
771	SB	US	BRO	1991
[f]*Pseudococcomyxa simplex* (Mainx) Fott				
MB 40/2	MB	BV	BEG	1996
Radiosphaera Pascher				
833	VV	US	BRO	1991
Stichococcus Nägeli.				
599	CB	US	BRO	1983
600	CB	US	BRO	1983
G 19/3	GH	BV	BEG	1996
631	II	US	BRO	1983
756	SB	AP	BRO	1991
MB 1/5	MB.	CH	BEG	1996
G 19/8	GH	BV	BEG	1996
G 41/1	GH	MS	BEG	1996
G 97/6	CR	BV	BEG	1996
MB 1/1	MB	CH	BEG	1996
MB 18/2	MB	EP	BEG	1996
MB 18/4	MB	EP	BEG	1996
MB 7A/1	MB	CH	BEG	1996
MB 37/7	MB	EP	BEG	1996
MB 64/1	MB	BS	BEG	1996
MB 42/5	MB	HY	BEG	1996
MB 63/2	MB	BV	BEG	1996
MB 40/4	MB	BV	BEG	1996
MB 40/8	MB	BV	BEG	1996
ISO 20	SI	AP	MAR	1994
ISO 19	SI	AP	MAR	1994
FB 100RR/L	SI	AP	MAR	1994
TH 48D	SI	AP	MAR	1994
JC 104RR/L	SI	AP	MAR	1994
JC 49D	SI	AP	MAR	1994
Tetracystis Brown and Bold				
647	CC	US	BRO	1985
763	SB	US	BRO	1991
798	SB	CR	BRO	1991
G 5/8	GH	MS	BEG	1996
G 8/3	GH	BV	BEG	1996
MB 37/3	MB	EP	BEG	1996
MB 49/3	MB	BS	BEG	1996

cont.

cont. Table 2.1

Strain number	[a]Location	[b]Habitat	[c]Isolator	Date of isolation
Trebouxia Puymaly				
839	VV	US	BRO	1991
G 95/7	CR	BV	BEG	1996
G 97/7	CR	BV	BEG	1996
MB 42/2	MB	HY	BEG	1996

[a]Location:

Antarctica

Maritime Antarctica

SI Signy Island (60°43′S, 45°38′W)
KGI King George Island (62°00′S, 58°00′W)

Continental Antarctica

LH Lützow Holm Bay (69°30′S, 39°30′E)
CA Casey Station area (66°17′S, 110°30′E)
CH Chapman Ridge (67°38′S, 160°57′E)
EP Edmonson Point (74°19′S, 165°08′E)
II Inexpressible Island (74°52′S, 163°40′E)
GH Granite Harbour (77°01′S, 162°30′E);
CB Cape Bird (77°14′S,166°23′E)
CC Cape Crozier (77°30′S, 169°20′E)
CR Castle Rock (77°48′S, 166°49′E)
SB Scott Base (77°50′S, 166°48′E)
VV Victoria Valley (77°23′S, 161°25′E)
MB Marie Byrd Land (77°30′S, 154°00′W)

New Zealand

CHCH Christchurch (43°30′S, 172°30′E)

Europe

AU Austria
Sw Switzerland

[b]Summary of habitats from which strains were isolated (partly from Broady, 1996).

Algal community	Habitat	Symbol
Lithophytic	On or within rock substrata	
Epilithic	On external, exposed surfaces	EP
Chasmoendolithic	Within fissures and cracks open to the rock surface	CH
Hypolithic	On the undersurfaces of diaphanous stones lying on soils	HY
Edaphic	On the surface of and within soils classified broadly as:	
	(i) mineral soil	MS
	(ii) biogenic soils enriched in nutrients by bird. and seal activity.	BS
	(iii) nature of soil unknown	US
	(iv) undersurface of lichen	UL
Epiphytic	On the living surface of bryophytes and lichens	
	(i) microscopic communities	BM
	(ii) visible crusts	BV
Cryophilic	Between ice crystals in surface layers of melting snow	CR
Airborne propagules		AP

[c]Isolators: BEG, A. Begum; BRO, Paul A. Broady; LIN, H. U. Ling; MAR, W. A. Marshall and M. O. Chalmers; OHT, S. Ohtani; PRI, E. G. Pringsheim; TSC, E. Tschermak-Woess; VIS, W. Vischer.
[d]Authentic (= type) strain
[e]Isolated from traces of soil on fresh vegetables brought to Scott Base from New Zealand
[f]Genera have been well described previously from Antarctica (Appendix 1) and therefore, not discussed in the text.

? Unknown.

clonal and unialgal cultures were stored, and for Petri plate cultures for initial isolation of strains and subsequent procedures (see section 2.4, 2.5, 2.6 and 2.7). Excess moisture was removed from the surface of medium in Petri plates by removing their lids and inverting in a 70^0C oven for 20 minutes.

All cultures were maintained at 15^0C, illuminated by fluorescent lamps (Philips TL 13W/33) delivering 50-150μmol $m^{-2}s^{-1}$ on a 16:8h light:dark cycle. This is termed "standard conditions" below.

2.2.2. *Isolation methods*

Isolations were made by standard microbiological methods of streak inoculation of agarised media. Inoculum for streak plates was derived from the PAB, CCAP and SAG culture collections, sample material and from moist plate enrichment culture.

A combination of moist plate enrichment culture (John, 1942; Lund, 1945) and culture in BBM were used for Antarctic samples. It was hoped that by using a combination of two methods for the qualitative examination of samples a large proportion of different strains of chlorophyte and xanthophyte algae would be recovered.

Moist plate enrichment cultures were established by placing portions of the samples in 9 cm wide Petri plates. Sterile water was added to moisten the sample without it being waterlogged. Flame-sterilised coverslips were then placed on the surface. The Petri plates were incubated in standard conditions. All were examined after two weeks incubation. An enriched growth of algae usually developed in the sample in the moist, warm and light conditions and on the undersides of the coverslips. The latter were removed from the plates and examined microscopically.

Sample materials and enrichment cultures were used as a source of inoculum for BBM agar cultures. Small quantities of soil and vegetation, e.g. a portion of bryophytes and lichen, with a drop of sterile distilled water were macerated and then spread over the agar plates with a wire inoculating loop. All cultures were incubated in the growth

cabinet under standard conditions as described in section 2.2.1. until visible colonies formed (~2 weeks). Discrete colonies from mixed communities were examined using a stereomicroscope and colonies which displayed differences in colour and texture (surface appearance) were chosen for further isolation. Selected colonies were streaked onto fresh BBM agar plates and incubated using the same conditions. Good growth appeared after two weeks incubations and were checked microscopically to see if they were unialgal.

All isolates were cleared from fungus and protozoa by removing individual colonies using finely pointed needles and then restreaking on BBM plates. This procedure was repeated until healthy, clean, unialgal cultures were obtained. Most cultures had low levels of bacterial contamination.

Single discrete colonies were removed for the inoculation on BBM agar slopes which were then maintained under standard conditions as a culture collection.

In addition, strains from other culture collections, PAB, CCAP and SAG, were re-isolated and cleaned using techniques described above.

All cultures are held by Paul Broady at University of Canterbury.

2.3. Morphology

2.3.1 Light microscopy (LM) studies

a) Equipment

Drawings at magnifications up to 2000x, using a 100x oil immersion objective combined with a 2x magnifier and 10x eyepieces were made using a *camera lucida* attachment. An Olympus BX 50 and Olympus BH2-RFCA were used for bright field microscopy and for Nomarski DIC (Differential Interference Contrast) respectively. These were fitted with photomicrographic systems (PM-10AK and C-35AD-4

respectively). Photographs were taken on Ilford Pan F (50 ISO) black and white film, using Nomarski DIC illumination, to show characteristic features, such as chloroplasts and pyrenoids.

b) Observation of morphological and reproductive features

Morphological and reproductive features of strains at exponential and stationary phases of growth were observed at regular intervals (14-21 days regarded as young and 60-80 as old cultures) for compilation of life-cycle information.

Specimens were mounted in a drop of distilled water on a glass slide and covered with a coverglass. The coverglass was usually ringed with nail varnish to retard drying of the preparation.

Morphological features. Measurements of cells were taken with a calibrated oculometer fitted within an eye-piece of the microscope. The following microscopical features were examined:

- Morphology
  - size
  - unicells or tetrads or filaments
  - cell shape, i.e. spherical/ellipsoidal/pyriform/irregular
- Cell wall characteristics
  - thickness
  - nature, i.e. smooth/warted
  - lens shaped thickenings
- Mucilage sheath around cells
- Contractile vacuoles characteristics
  - number
  - location
- Storage product characteristics

location

chemistry, e.g. starch, oil, etc.

- Nuclei characteristics

  number

  position

- Morphological changes with age, i.e. accumulation of carotenoid
- Chloroplast characteristics

  shape, i.e. cup-shaped/discoidal/spindle/ polygonal/spongy/lobed

  position, i.e. axile/parietal

  number in adult cells

- Pyrenoid characteristics

  nature, i.e. immersed/bulged/naked/surrounded by starch.

  number

  nature of starch sheath around pyrenoid, i.e. entire/perforated/lobed

Indian ink, 0.5% toluidine blue and 0.5% methylene blue solutions were added to fresh mounts to determine the presence or absence of gelatinous matrices and to clarify cell wall thickness. A dilute I_2KI (Lugol`s iodine; Archibald and Bold, 1970) solution was used to determine number and position of nuclei in both vegetative cells and zoospores.

In chlorophytes, I_2KI was used to investigate the nature of the starch sheath surrounding the pyrenoid. Cells were removed from a culture into a drop of dilute I_2KI solution on a slide. Pressure was applied to the coverglass in order to crush the cells until chloroplasts and cell walls were completely disrupted. This released intact pyrenoids with their starch sheaths stained blue-black. These were examined at 2000x magnifications and details of the structure of the starch sheath were noted. Details included whether the sheath was entire or comprised separate starch grains.

In addition, 0.1% Azocarmine-G (Sigma Chemical Co., A-0677) was used for staining pyrenoids in chloroplasts of xanthophytes (Ettl and Gärtner, 1995).

Cell length and width of the eight strains of *Stichococcus* were measured using a calibrated oculometer. Fifty random cells were selected for measurements. Cell outlines were made using a *camera lucida*.

Single factor analysis of variance (ANOVA) was carried out on length and width data of each strain of *Stichococccus*. An unplanned comparison among means was carried out using the Tukey-method (Sokal and Rohlf, 1981) to calculate the minimum significant difference (MSD) at the 95% level of confidence. All measurement data were analysed using SAS computer package.

Reproductive features. The following reproductive features were examined:

- Sporangium characteristics
  - size
  - shape
  - number of spores
  - method of release of spores, i.e. by rupture/ gelatinization of sporangium wall
- Aplanospore characteristics
  - size
  - shape
  - number of chloroplasts
- Akinete characteristics, i.e. shape
- Vegetative division characteristics, i.e. presence or absence
- Zoospore characteristics
  - size
  - shape, i.e. spherical/pyriform/ellipsoidal
  - behaviour after release from zoosporangium, i.e. shape remains the same or changes following cessation of motility
  - number of chloroplasts
  - position of pyrenoid, i.e. anterior/median/posterior
  - presence or absence of stigma
  - position of stigma, i.e. anterior/median/posterior
  - presence or absence of contractile vacuoles

shape of papillae, i.e. papillate/bifurcated
number of flagella
type of flagella, i.e. equal/unequal
length of flagella

To obtain sufficient numbers of zoospores for study, fresh liquid BBM was poured onto a seven day culture on agarized medium. These cultures were kept at standard condition and observed at regular intervals (14, 21, 28 and 35 days). Mass liberation of zoospores was usually seen in 14-21 day cultures.

A dilute I_2KI solution was used to determine the number and length of flagella of zoospores.

2.3.2. Transmission electron microscopy (TEM)

a) A comparison of the effect of different temperatures on primary fixation

Room temperature fixation. Two week old cultures of strains of *Botrydiopsis, Chlorellidium, Botryochloris* and *Stichococcus* grown under standard conditions (as described in section 2.2.1), were fixed and stained for examination using TEM. 3% glutaraldehyde (w/v) with 0.75 M sodium phosphate buffer at pH 7.2 was poured onto colonies on the plate surface and left for two hours at room temperature (approx. 20^0C).

Hot fixation. Two week old cultures of strains of *Botrydiopsis* and *Chlorellidium* were fixed in 3% glutaraldehyde, in 0.75 M sodium phosphate buffer at pH 7.2 at each of 40^0, 60^0 and 80^0C for four hours (Fineran, 1994). Cells fixed at room temperature were used as the control against which the efficiency of hot fixation was assessed.

b) Post-fixation and dehydration

Small agar blocks covered with growth of *Botrydiopsis, Chlorellidium* and

Botryochloris were cut out of the fixed material and coated with molten 2% agar at 70^0C which was allowed to solidify at room temperature (approx. 20^0C). For *Stichococcus*, the cells were gently scraped off the agar into Eppendorf tubes (1.5 ml) and centrifuged (Pickett-Heaps, 1974) at 3000 rpm for two minutes to obtain sufficient number of cells. The pellet of cells was embedded in 2% agar and cut into small blocks (4x4x5 mm) and stored at 4^0C in 0.75 M sodium phosphate buffer overnight. These were then rinsed three times in buffer for 10 min each and flooded with 1% osmium tetroxide (OsO_4) for two hours, followed by a final rinse in buffer for 10 min. Dehydration was carried out in a series of aqueous acetone solutions of increasing concentration of acetone from 20, 40, 60, 80 to 100%, for 10 minutes each, with a final three rinses in 100%.

c) Infiltration and embedding

Blocks were infiltrated with a solution of Spurr`s resin and 100% acetone in a ratio of 1:2 for two hours, followed by a 2:1 resin:acetone solution overnight. Blocks were rotated at *ca.* 1 rpm during infiltration. Infiltrated material was then embedded in 100% Spurr`s resin, without rotation and oven dried at 70^0C overnight.

d) Sectioning and staining

Small blocks containing cells were cut out of the resin, trimmed and sectioned using a LKM Bromma 2128 Ultratome. Ultra-thin sections (80-100 nm) were cut using a diamond knife, and mounted on copper grids (200 mesh). Sections were double-stained, first for 10 mins with 1% uranyl acetate in 50% ethanol, followed by five minutes with 2% lead citrate (2 g dissolved in 100 ml water).

e) Photography

A JEOL JEM-1200EX electron microscope set at an acceleration voltage of 80kV was used to examine the sections. All photographs were taken at 7,500x to 50,000x magnifications using Kodak Electron Microscope Film 4489.

2.3.3. Scanning electron microscopy (SEM)

The delicate surface features of algae are clearly revealed by SEM technique (Rosowski *et al.*, 1975, 1981; Rosowski and Langenberg 1994). Here, SEM was used to observe surface features of cell walls of selected isolates of *Botrydiopsis* (strains 836 and 896). Cells scraped from the surface of a two week old culture grown under standard conditions were suspended in distilled water. A clean coverglass (22x22mm) was mounted on an aluminium stub with a thin layer of glue (carbon paint, catalogue no. IA003, ProSci Tech, QLD). A drop of the suspension was placed on the coverglass and dried at room temperature (approx. 20^0C) overnight.

Algae were sputter coated with gold for two minutes using a Polaron E5000 (UK) coating unit.

Specimens were examined at magnifications of 4,000-8,200x with a Leica S440 scanning electron microscope operated at 20 kv. Photography used Ilford FP4 (ASA 125) black and white film.

2.3.4. Data analysis

Cluster analysis was performed using a presence/absence data matrix of morphological characteristics transformed into binary (1/0) data (Buchheim *et al.*, 1990). A minimum of 60 morphological characters are required to obtain stable classification (Sneath and Sokal, 1973). Multistate characters were converted into several independent characters to fulfill this requirement and then coded as 1/0, i.e. binary characters. Data was analysed using the S-Plus statistical program (Mathsoft Inc., 1995; Appendix 2). Numerical affinity between operational taxonomic units (OTU) was determined using general coefficient of similarity, S_j (Sokal and Sneath, 1963; Everitt, 1974). Results were expressed as a similarity matrix (Tables 3.3, 3.4). Cluster analysis was carried out using the UPGMA (Unweighted Pair Group Method with Arithmetic averages, or,

"average-linkage") method (Everitt, 1974; Sokal and Sneath, 1963). The results were expressed as a dendrogram based on genetic distance and this was used to interpret relatedness amongst the strains of *Botrydiopsis* and *Chlorellidium.*

2.4. Isozyme electrophoresis

Enzyme electrophoresis technique was used to investigate the genetical variations and/or relatedness between strains from: i) different regions, i.e. Antarctica, Europe, New Zealand, ii) different localities within Antarctica, iii) different samples from the same locality in Antarctica, and iv) different isolates from the same sample. In addition, different isolates from the same clone were used to check the validity of the method.

2.4.1. Testing of buffer systems

Four different electrophoretic buffer systems (Table 2.2) with two different extraction buffers (Table 2.3) were tested for their ability to detect enzyme activity. The only successful system was lithium borate electrophoretic buffer combined with extraction buffer B. No band was detected in the other three electrophoretic buffers when combined with either extraction buffer A or B. Extraction of a minimum of 0.01 g of alga with 200 µl extraction buffer produced enough enzyme for detection. From each extracted algal sample, a minimum of 33 µl of supernatant was needed for electrophoresis.

2.4.2. Extraction of proteins

Three week old cultures of *Botrydiopsis, Botryochloris* and *Chlorellidium* grown under standard conditions, were used for isozyme investigation. The algal mass was scraped from the agar surface and ground in liquid nitrogen with a mortar and pestle until cells were disrupted. The resulting paste was suspended in 200 µl of extraction buffer (Table 2.3) in a 1.5 ml centrifuge tube. Cell debris was pelleted by centrifugation at 10,000 rpm for 15 min at 0^0C. The supernatant was decanted and stored at -80^0C. It was assumed that extracts stored for three months could be used without loss of enzyme

activity and without detectable changes in isozyme band mobility and intensity as found by Buchheim *et al.* (1990).

Table 2.2. Different electrophoretic buffer systems tested for their ability to detect enzyme activity.

Electrode buffer	Gel buffer	Current (mA) or Voltage (V)	Reference
Tris-citrate (pH 8.0) (16.35 g Tris, 6.10 g citric acid monohydrate, 1.00 litre water)	Tris-citrate (pH 8.0) (electrode buffer diluted 1:19)	48 mA	Soltis *et al.* 1983
Lithium borate (pH 8.3) (1.20 g $LiOH.H_2O$, 11.89 g boric acid, 1.00 litre water)	Lithium borate (pH 8.3) (electrode buffer mixed 1:9 with solution of 5.45 g tris, 1.28 g citric acid monohydrate and 1.00 liter water)	30 mA	[a]Selander *et al.* 1986
Amine-citrate/Morpholine (pH 6.1) [8.4 g citric acid monohydrate, 17ml N-(3 aminopropyl)-morpholine]	Amine-citrate/Morpholine (pH 6.5) (electrode buffer diluted 1:19)	48 mA	Murphy *et al.* 1996
Borate (pH 8.0) (18.178 g boric acid, 2.4 g NaOH, 1.00 litre water)	Tris-citrate (pH 8.65) (9.206 g tris, 1.050 g citric acid monohydrate, 1.00 litre water)	200 V	Thomas and Brown 1970

[a]The pH of electrode buffer and amount of ingredients of gel buffer have been modified by H. Chapman (University of Canterbury, pers. comm.) to improve the enzyme activity.

Table 2.3. The two extraction buffer systems examined for their ability to resolve enzymes.

Extraction buffer	pH	Reference
A. 0.438 g NaCl, 0.071 g Na_2PO_4, 50 ml water	7.5	Thomas and Brown, 1970
B. 50 ml 0.2M Tris HCl, 0.0216 g boric acid, 0.0142 g sodium sulphate, 0.495 g sodium ascorbic acid, 0.0744 g [a]EDTA, 0.0342 g sodium diethyldithio carbamic acid, 0.25 g PVP-40T, 50µl 2 mercaptoethanol	7.5	Buchheim *et al.*, 1990

[a]EDTA, Ethylenediaminetetraacetic acid

2.4.3. Gel preparation

The method used in this study was identical to that used by Murphy *et al.* (1996). A gel mold (electrophoresis buffer tray) was used for horizontal starch gel electrophoresis. An 11% w/v gel of starch in electrophoretic buffer was made in the following manner. 33 g of starch was suspended in 100 ml of gel buffer. A further 200 ml of buffer was heated in a microwave oven until boiling. Solutions were combined and heated for a further 2 min until boiling with stirring every 15 sec. The starch suspension was aspirated for 20 sec to remove air bubbles and then immediately poured into a gel mold. If any air bubbles remained in this suspension, they were removed within 1 min using a Pasteur pipette. The gel was allowed to cool to room temperature (approx. 20^0C) for two hours. It was covered in plastic film to prevent desiccation and stored at 4^0C. All gels were used within 24 hours of preparation.

2.4.4. Gel loading and electrophoresis

A gel comb was inserted vertically into the gel 2 cm from the negative pole to make wells. Up to 22 isolates were loaded per gel, by inserting 4x10 mm segments of Whatman cellulose filter paper (no. 1003185), saturated with the extracted proteins, into each gel comb slot. Pieces of filter paper dipped into 0.1% bromophenol blue were loaded into both terminal slots to act as markers for the buffer front during migration. Electrophoresis was conducted in a refrigerator at 4^0C and gels were additionally cooled by placing an ice pack on top of the gel. A constant current of 30 mA (for lithium borate buffer system) was applied until the blue dye front had migrated off the gel.

2.4.5. Gel slicing and staining

Following electrophoresis gels were removed from the buffer tray and cut into 3-4 pieces (1-2 mm thick) using a bow slicer. The slices were separated and placed in individual staining trays with the sliced surface of the gel facing upward. Each slice was incubated individually at 37^0C in the dark, in various staining solutions until bands became visible. Gels were stained (see Table. 2.4 for stain recipes) for nine different

enzymes: phosphoglucose isomerase (PGI), leucine amino peptidase (LAP), glucose-6-phosphate dehydrogenase (G6PDH), shikimate dehydrogenase (SKDH), esterase (EST), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM) and hexokinase (HK). Only four systems, PGI, 6PGD, LAP and SKDH consistently produced bands. For each strain, 3-4 replicate starch gels were run for each enzyme system in order to check consistency of results.

2.4.6. Data recording and analysis

Photographs (Kodak T-Max, black and white, ESO 100) were taken immediately after the banding patterns appeared, but neither all bands nor the intensity observed on the freshly stained gel were easily observed in these. Therefore, bands visible to the eye were traced on to A4 size plastic sheet.

Each isozyme band was scored as a binary character, i.e. presence or absence of the band in a particular strain (e.g. Chinain *et al.*, 1997; Buchheim *et al.*, 1990). It was decided not to score bands as allele frequency amongst strains as this is subject to modification by genetic drift and/or selection (Mickevich and Johnson, 1976).

Sexual reproduction is unknown in *Botrydiopsis, Botryochloris* and *Chlorellidium.* Formal karyological analysis is required to know the chromosome status which is beyond the scope of the present investigation. If the assumption is made that each band is derived from a haploid cell then these represent the products of a presumptive locus. These bands can then be treated as characters for systematic analysis (Buth, 1984; Buchheim *et al.,* 1990). We adopted this assumption for our analysis and identified each isozyme band as a binary character with presence or absence (1/0) as the equally weighted states of the character.

Binary data was analysed using the S-Plus statistical program (Mathsoft Inc., 1995; Appendix 2). Numerical affinity between operational taxonomic units (OTU) was

Table 2.4. [a]Stains used for visualizing algal enzymes following electrophoresis.

Enzymes	[b]Staining solution						
	Substrate	Enzyme	Co-enzyme	[c]Buffer	Salt	Dye	Catalyst
Leucine aminopeptidase (LAP)	[d]L-leucyl-beta naphthylamide HCl (0.05 g) N-N-dimethylforamide (2 ml)	-	-	0.2M tris maleate (pH 5.3)	10% $MgCl_2$ (1 ml)	Fast black K salt (0.07 g)	-
Shikimate dehydrogenase (SKDH)	Shikimic acid (0.05 g)	-	[e]NADP (0.010 g)	0.2M tris-HCl (pH 8.5)	10% $MgCl_2$ (0.05 ml)	-	[e]MTT (0.015 g) PMS (0.003 g)
Glucose-6-phosphate dehydrogenase (G6PDH)	Glucose-6-phosphate (disodium salt, 0.1 g)	-	[e]NADP (0.02 g)	0.2M tris-HCl (pH 8.00)	10% $MgCl_2$ (0.5 ml)	-	[e]MTT (0.01 g) PMS (0.002 g)
Phosphoglucose isomerase (PGI)	Fructose-6-phosphate (0.080 g)	[f]Glucose-6-phosphate dehydrogenase (1.00 ml)	[e]NADP (0.010 g)	0.1M tris-HCl (pH 8.5)	10% $MgCl_2$ (0.05 ml)	-	[e]MTT (0.015 g) PMS (0.003 g)

[a] Source of recipe for stain solutions is Soltis *et al.* (1983). The amount of ingredients of stain solutions for LAP, SKDH and PGI have been modified by H. Chapman (University of Canterbury, pers. comm.) to improve the resolution of algal enzymes. MTT, (3-[4,5-Dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide); PMS, Phenazine methosulfate; NADP, ß Nicotinamide adenine dinucleotide phosphate.

[b] Ingredients were mixed with buffer solutions.

[c] Dissolve tris in 50 ml distilled water for all enzymes except G6PDH for which 100 ml distilled water is used.

[d] Dissolve 0.05 g L-leucyl-beta naphthylamide HCl in 2 ml N-N-dimethylforamide and mix with 0.2M tris maleate buffer.

[e] Dissolve PMS, MTT and NADP in 0.05 ml 10% $MgCl_2$ for PGI, G6PDH and SKDH and mix with 0.2M tris-HCL buffer except G6PDH (0.1M tris-HCL).

[f] Add 1 ml glucose-6-phosphate dehydrogenase at the end of preparation of staining solution.

determined using general coefficient of similarity (Sokal and Sneath, 1963; Everitt, 1974). This coefficient (S_j) is calculated with the formula: $S_j = a / (a+u)$, where a is the number of matches, i.e. shared bands, and u is the number of non-matches between two OTUs. The closer S_j is to 1.00 the more similar or more related two isolates are considered to be. Results were expressed as a similarity matrix (Tables 3.8 and 3.9). Cluster analysis was carried out using the UPGMA (Unweighted Pair Group Method with Arithmetic averages, or, "average-linkage") method (Everitt, 1974; Sokal and Sneath, 1963). The results were expressed as a dendrogram based on genetic distance and this was used to interpret relatedness amongst the strains of *Botrydiopsis* and *Chlorellidium*.

2.5. Carotenoid pigment analysis

2.5.1. Pigment extraction

Three week old cultures of *Botrydiopsis* and *Chlorellidium* grown under standard conditions were used for analysis of pigment by HPLC (high performance liquid chromatography). The method used was adapted from Heukelem *et al.* (1994). Whittle and Casselton (1969) and Downes *et al.* (1993) warned that storing extracts of algae deep frozen for periods up to 15 h could lead to degradation of chlorophylls and isomerisation of xanthophylls. Downes *et al.* (1993) found mild sonication of material in 90% acetone minimised chlorophyll *a* breakdown and dramatically improved the extraction efficiency of chlorophyll *a* and carotenoids.

Pigment extractions were performed on freshly harvested cultures. Cells scraped from the surface of agarised culture medium were placed in 1.5 ml cold (-20^{0}C) 90% acetone in Eppendorf tubes in a dimly lit room. All samples were stored deep-frozen in the dark at -90^{0}C overnight and allowed to warm to room temperature for 30 minutes prior to analysis. Samples were then homogenised with a grinder (Ultraturex, model T8.01, 100W, IKA Labortechnik, Germany) for 15-30 seconds followed by probe sonication for 15-30 seconds (10 pulses) using a model W225 sonicator (Ultrasonics Inc., USA). Samples were then left to extract in the dark in a refrigerator (4^{0}C) for 2 hours. Each

extract was then filtered through a 0.45 μm pore size HPLC syringe cartridge (Gelman Acrodisc 3 CR PTFE) directly into a 2 ml amber crimp-top vial and placed in a Shimadzu CTO-6A column oven held at 2 to 3^0C until injection.

2.5.2. HPLC analysis

Subsamples of extract (100μl) were injected into a Shimadzu HPLC system comprising the following modules: an SIL-6B auto-injector, two LC-6A solvent pumps and a SCL-6B gradient controller. The pigments were separated on a C18 reverse-phase, Vydac 201TP, 300 A^0 pore size, 5 μm particle size, 4.6 x 250 mm column (Separations Group, Hesparia, California). The pigments were then measured with a Shimadzu SPD-6AV variable wavelength UV-visible detector set at 440 nm connected in series with a Hewlett Packard 1046A programmable fluorescence detector set at an excitation wavelength of 430 nm and emission wavelength of 665 nm. HPLC was conducted at 60^0C in a Shimadzu CTO-6A column oven.

Subsamples for pigment identification were separated on the same HPLC system coupled to a Shimadzu SPD-M6A photodiode array detector. The binary solvent system of Mantoura and Llewellyn (1983) was used with the exception that tetrabutylammonium acetate was omitted from Solvent A (Zapata *et al.*, 1987). The solvents were formulated as follows: solvent A was methanol:0.5M ammonium acetate, 80:20; solvent B was methanol:acetone, 80:20. Flow was at 1.5 ml min^{-1} throughout the 30 min separation.

2.5.3. Pigment identification

Identification was based on retention times, spectra and known pigment composition of yellow-green algae. The pigment peak areas were converted to concentrations using the published extinction coefficients for identified peaks provided by Jeffrey *et al.* (1997). The unidentified carotenoid peaks were converted to concentrations using an approximate extinction coefficient calculated by Mantoura and Llewellyn (1983).

Amounts of other pigments were calculated by E1% values given by Jeffrey *et al.* (1997).

3. RESULTS

3.1 Introduction

In the present study 44 morphotypes from 14 genera of chlorophyte and three genera of xanthophycean algae have been described from cultures. Morphology of both chlorophyte and xanthophycean algae, and isozyme and pigment characteristics of xanthophyte algae are described in the following sections.

The classification of Hoek *et al.* (1995) has been adopted. They placed all xanthophycean algae in Heterokontophyta and all "green" algae in Chlorophyta. This recent classification is based on zooid characteristics, storage products, pigment composition and chloroplast features. The taxonomic work of Ettl and Gärtner (1995) was used for identification to genus and species.

3.2 Xanthophyceae

The Heterokontophyta constitutes a natural group now recognised to contain at least nine algal classes. Seven orders are usually recognised in the class Xanthophyceae (Hoek *et al.,* 1995). Most coccoid unicells belong to the order Mischococcales (Ettl and Gärtner, 1995; Hoek *et al.,* 1995). This order has the most species of any order in the Xanthophyceae (Hibberd, 1982). This investigation has concentrated on three genera in this order, namely *Botrydiopsis, Botryochloris* and *Chlorellidium.* Ettl and Gärtner (1995) placed *Botrydiopsis* in the family Botrydiopsidaceae, and both *Botryochloris* and *Chlorellidium* in the Botryochloridaceae.

This study has recognised nine morphotypes of these three genera. Strains assigned to each morphotype are shown in Table 3.1. This also indicates minor interstrain differences. The following sections provide detailed descriptions of morphology and isozyme characterictics of each morphotype, and pigment composition of morphotypes of *Botrydiopsis* and *Chlorellidium.*

Table. 3.1. Assignment of strains to different morphotypes of *Botrydiopsis*, *Botryochloris* and *Chlorellidium*, indicating minor morphological differences between strains as observed in LM and TEM.

Morphotypes	Strains[1]
Botrydiopsis	
B1	645^{ax}, 894^{ax}, 829^{ax}, 801^{bx}, $G41/6^{cx}$, WI/I^{dx}
B2	886^{ax}, $G12/1^{ax}$, 724^{ax}, 896^{ax}, 895^{bx}, 897^{cx}
B3	485
B4	G94/1
B5	836
B6	837^{ax}, $G19/2^{ax}$, $G24/8^{ax}$, $G27/1^{ax}$, $G31/5^{ax}$, $G9/8^{bx}$, $G99/2^{cx}$
B7	877^{ax}, 638^{ay}
B8	864^{ax}, $G27/2^{bx}$
B9	908
Botryochloris	
BC1	G19/1
Chlorellidium	
C1	597^{ax}, 757^{bx}, 758^{cx}, 898^{cx}, $G28/7^{dx}$, $G31/6^{dx}$
C2	785^{ax}, 871^{ay}

[1]Refer to Table 2.1 for origin of strain.
Within a morphotype, strains assigned the same superscript letter are identical, those with different letters have minor morphological differences. a-d, refer to LM characteristics. x, y, refer toTEM characteristics. These minor differences are described in the text.

3.2.1 Morphology

a) A comparison of the effect of different temperatures on primary fixation for TEM

The effect of temperature on the quality of images is shown in Table 3.2 and Fig. 1. Out of 13 strains of *Botrydiopsis*, *Botryochloris* and *Chlorellidium*, only three strains were well-fixed at 20^0C with good images of ultrastructural features being produced. The single strain each from *Botryochloris* and *Botrydiopsis* was poorly and moderately fixed

respectively at this temperature. Of the remaining eight strains, good fixation occurred in three at 40^0C, six at 60^0C and five at 80^0C. However, hot fixation did not produce identical results within a morphotype. Strains of each morphotype B7, C1 and C2 had different responses. For example, in morphotype B7, strain 877 fixed well at 60^0 and 80^0C but 638 fixed poorly.

Table. 3.2. A comparison of fixation quality at different temperatures for TEM of *Botrydiopsis, Botryochloris* and *Chlorellidium.*

Strain no.	Morphotype	[a]Pyrenoid	[b]Fixation temperature (^{0}C)			
			20	40	60	80
Botrydiopsis						
894	B1	-	I	ND	ND	ND
896	B2	-	I	I	I	I
836	B5	-	III	III	I	III
837	B6	+	III	III	III	I
877	B7	+	III	I	I	I
638	B7	+	III	I	III	III
864	B8	+	III	III	I	I
908	B9	+	II	ND	ND	ND
Botryochloris						
G19/1		-	III	ND	ND	ND
Chlorellidium						
898	C1	-	III	I	I	III
597	C1	-	III	III	I	I
871	C2	+	I	I	I	I
785	C2	+	III	III	I	I

[a] +, presence of pyrenoid; -, absence of pyrenoid.

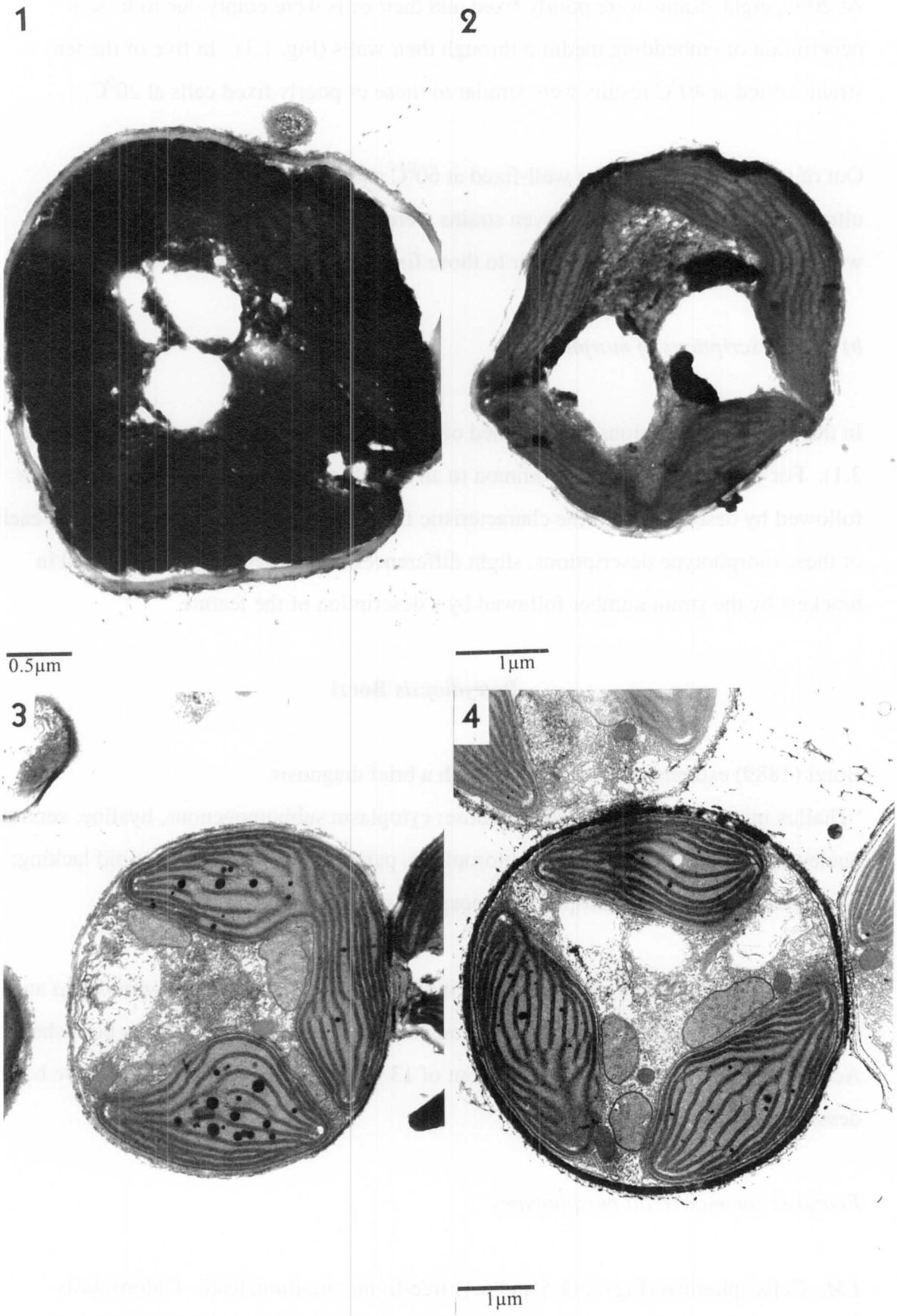
[b] two replicate treatments at each temperature produced identical results.

Key: I, good fixation; II, moderate fixation; III, poor fixation; ND, no data.

Fig. 1. An example (*Chlorellidium,* morphotype C2, strain 785) of the effects of different temperatures on primary fixation for TEM.

1, fixation at 20^0C for 4 h resulting in poor fixation. 2, fixation at 40^0C for 4 h resulting in poor fixation. 3, fixation at 60^0C for 4 h resulting in good fixation. 4, fixation at 80^0C for 4 h resulting in good fixation

Scale in 2 also applies to 3.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0.5µm
1µm
3
4
1µm

At 20^{0}C, eight strains were poorly fixed and their cells were empty due to lack of penetration of embedding medium through their walls (Fig. 1.1). In five of the ten strains tested at 40^{0}C results were similar to those of poorly fixed cells at 20^{0}C.

Out of ten strains, eight were well-fixed at 60^{0}C and overall visual quality of ultrastructure was increased. Seven strains were well-fixed at 80^{0}C. Appearance of well-fixed cells at 80^{0}C was similar to those fixed at 60^{0}C (Figs.1.3, 4).

b) Descriptions of morphotypes

In this section descriptions are provided of the morphotypes of all three genera (Table 3.1). For each genus, features common to all morphotypes are described first. This is followed by descriptions of the characteristic features of each morphotype. Within each of these morphotype descriptions, slight differences in particular strains are noted in brackets by the strain number followed by a description of the feature.

Botrydiopsis **Borzi**

Borzi (1889) established *Botrydiopsis* with a brief diagnosis.
"Thallus microscopic, unicellular, globose; cytoplasm subhomogenous, hyaline; central nucleus; thin cell wall; numerous chloroplasts, parietal, disc-shaped, pyrenoid lacking; propagation by gonidia, zoospore, hypnospore or zygospore."

A pyrenoid in each chloroplast is known in two species, *Botrydiopsis pyrenoidosa* and *B. callosa* (Trenkwalder, 1975), but an emended diagnosis has not yet been published. According to Ettl and Gärtner (1995), out of 13 species of *Botrydiopsis,* five have been described from soil.

Features common to all morphotypes

LM. Cells spherical (Figs. 5.4-5 and 6.1) free-living, multinucleate. Chloroplasts numerous, parietal, discoid or fusiform. Oil globules numerous, scattered throughout

the cell. Reproduction by aplanospores or zoospores. Zoospores with two unequal flagella.

TEM. Chloroplast lamellae consisting of 3 parallel thylakoids. Girdle lamella present. Chloroplast envelope with double membrane and bounded by a chloroplast endoplasmic reticulum (CER), continuous with the outermost membrane of the nuclear envelope (Fig. 7.4). Darkly stained lipid droplets occur randomly between the lamellae. Vesicular structure (i.e. periplastidial network, Hibberd, 1980) appears between chloroplast envelope and CER (Fig. 17.5). Genophore present in a region of less dense matrix between girdle lamellae and the ends of the internal lamellae (Fig. 20.5). A pair of centrioles lie near the nucleus-plastid complex. Mitochondria scattered, with tubular cisternae. Golgi bodies lie against the nuclear envelope.

Zoospore formation indicated by i) chloroplasts coming to lie against each nucleus, these arranged around the periphery in larger zoosporangia (Fig. 14.3), ii) appearance of eye spot in chloroplast (Fig. 7.1), iii) bases of flagella coming to lie against the chloroplast envelope facing the plasma membrane.

Morphotype B1 (B. constricta)
(Figs. 2.1-15; 3.1-17 and 4.1-8)

LM. Cells spherical to broadly ellipsoidal (Figs. 2.1-4, 6 and 4.1-2), single, up to 49 μm diameter, but mostly about 13 μm diameter. Cell wall smooth, thin in young cultures but up to 4 μm thick and stratified, occasionally with one or several lens-shaped local thickenings up to 2.5 μm wide (Figs. 2.5 and 4.3) in old cultures, without superficial granules. Mucilage sheath present in old cultures (Fig. 2.9). Chloroplasts more than 20 in adult cells, spindle to discoidal to polygonal, lacking pyrenoids (Figs. 2.1-6 and 4.1-2). Vacuoles large, scattered.

Sporangia up to 33 μm diameter (Figs. 2.7-8, 10 and 4.6), containing two to numerous (>20) spores. Sporangium wall smooth, thin, occasionally up to 4 μm thick and stratified in old cultures. Spores released by rupture of sporangium wall (Fig. 2.11),

Fig. 2. *Botrydiopsis* morphotype B1.

1-2, young cells. 3-6, spherical to ellipsoidal cells showing discoidal to polygonal chloroplasts which lack pyrenoids. 5, single cell with lens-shaped thickening of cell wall. 7, ellipsoidal zoosporangium. 8, zoosporangium with a thick, stratified wall and showing a distinct stigma in each chloroplast. 9, ellipsoidal cell with mucilage sheath. 10, large, spherical autosporangium. 11, spores released by rupture of sporangium wall. 12, persistent sporangium wall fragment adhering to spore in strain G41/6. 13-14, zoospores. 13, pyriform to amoeboid zoospores each with a single chloroplast. 14, larger zoospores found in strains 801 and WI/1. 15, spherical to ellipsoidal aplanospores.

Scale bar is 10 μm.

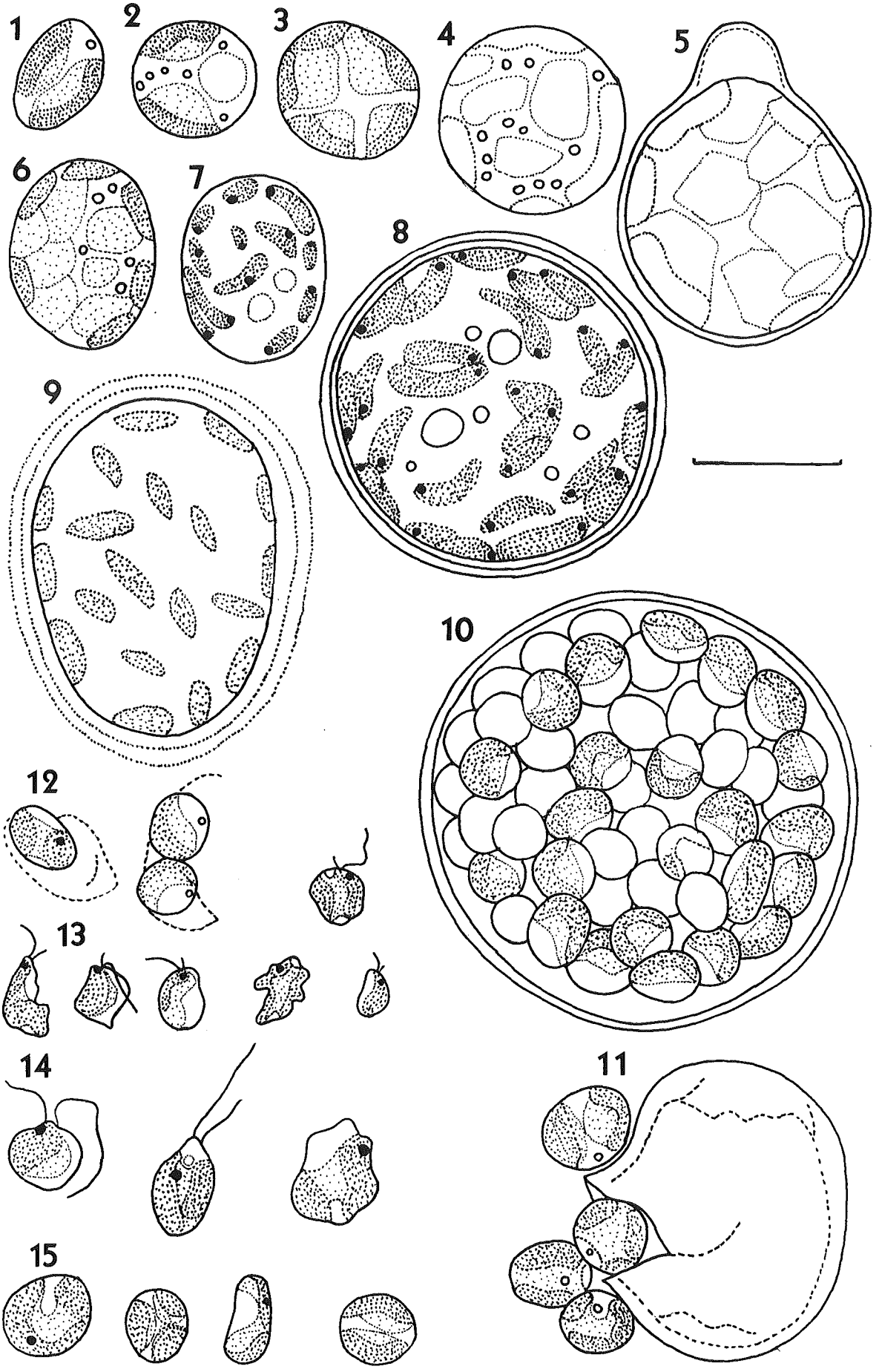
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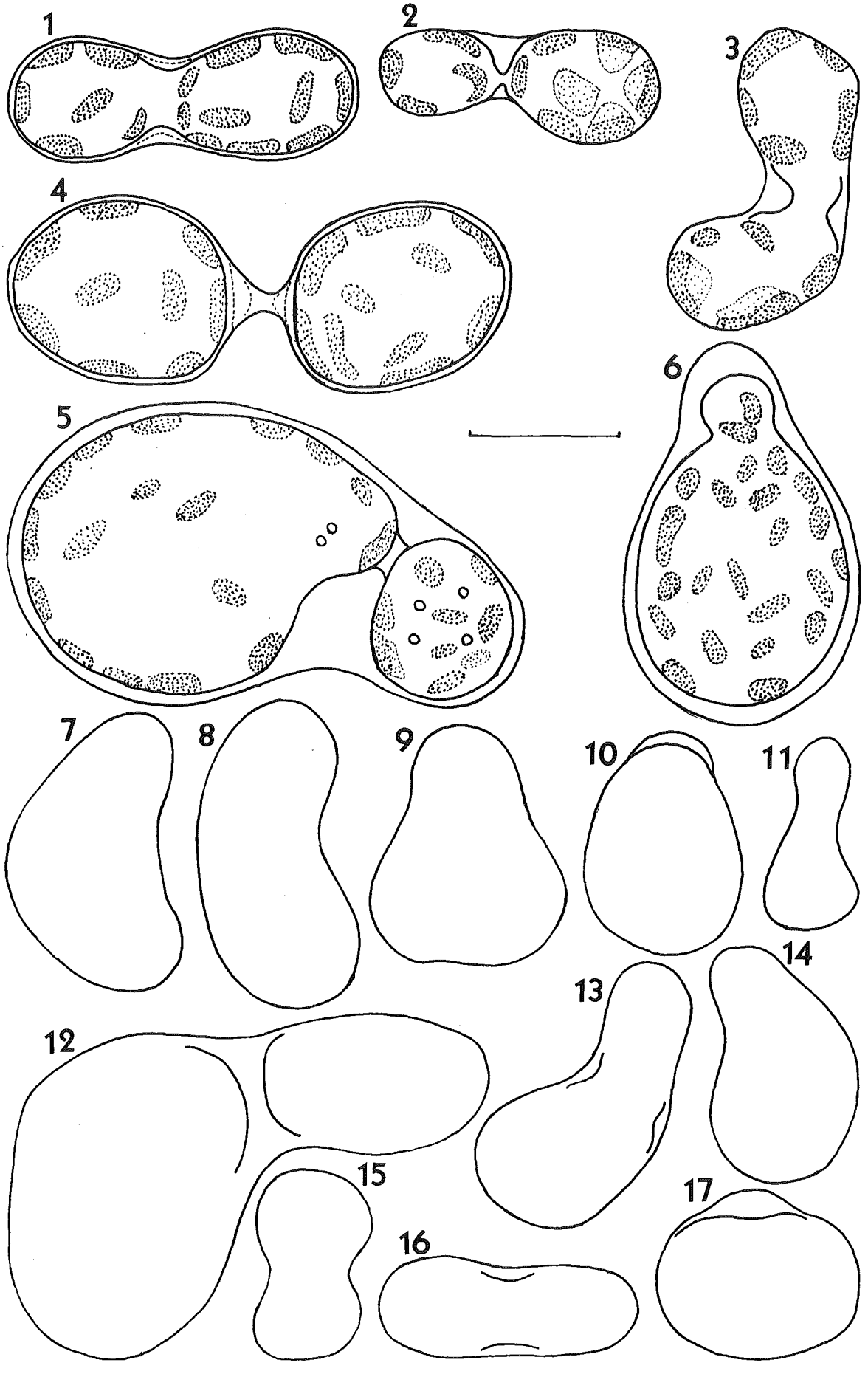
Fig. 3. *Botrydiopsis* morphotype B1.

1, early vegetative division. 2-4, late stages in division. 5, late stage of "budding" division. 6, constriction and formation of small daughter cell. 7-17, outlines of cells from an actively dividing culture, showing cell wall thickenings when present.

Scale bar is 10 μm.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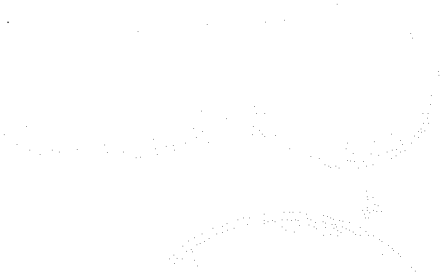
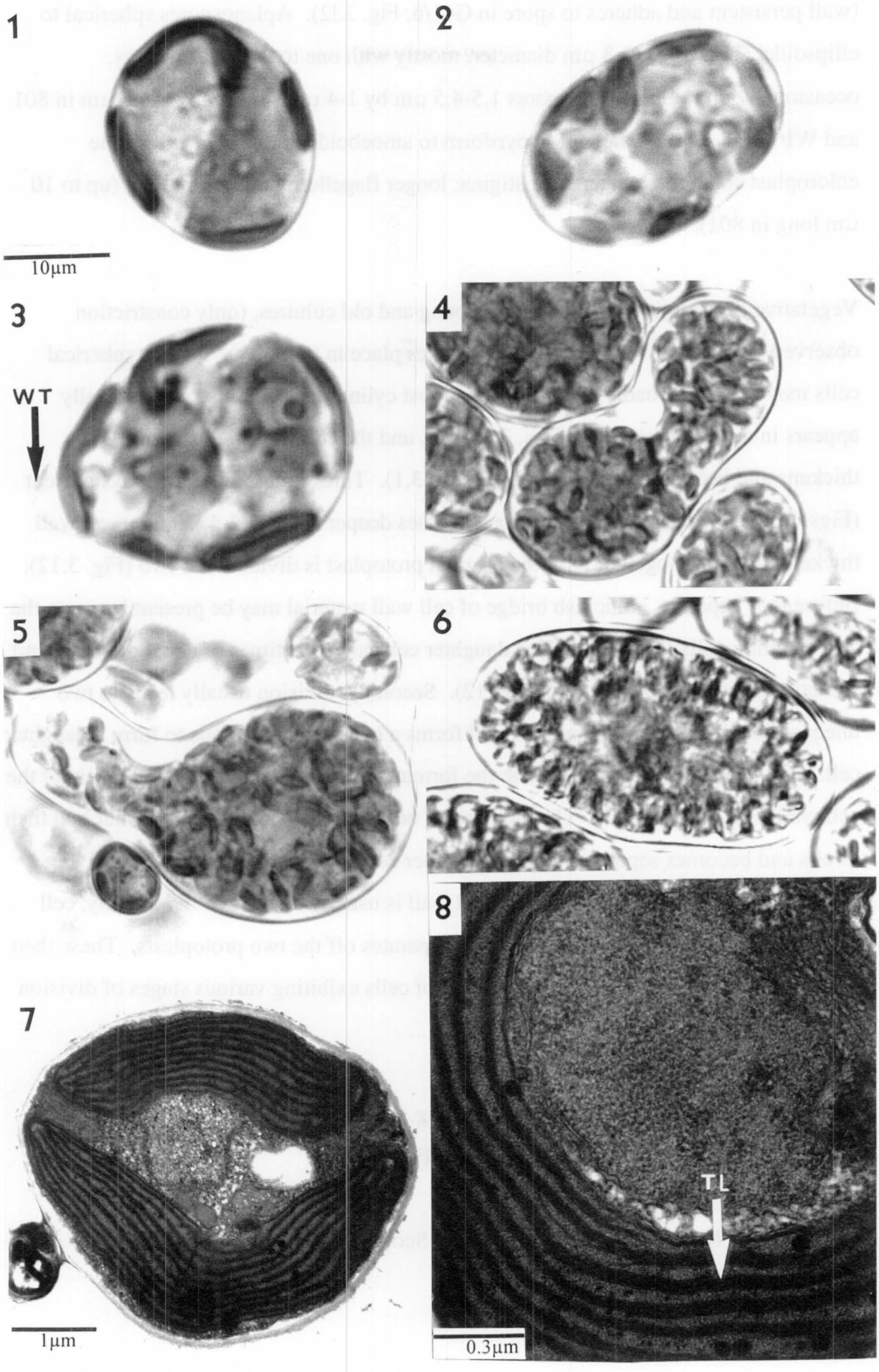


Fig. 4. *Botrydiopsis* morphotype B1, LM (1-6) and TEM (7, 8).

1, adult cell showing discoidal chloroplast lacking pyrenoid. 2, ellipsoidal cell. 3, cell with lens-shaped cell wall thickening (WT). 4, late stage in cell division and transforming into a zoosporangium. 5, late stage of "budding" division. 6, zoosporangium containing numerous zoospores. 7, cell showing chloroplasts lacking a pyrenoid. 8, region of a chloroplast showing thylakoid lamellae (TL) and lack of pyrenoid.

Scale in 1 also applies to 2-6.


1
10µm
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WT
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6
7
1µm
8
TL
0.3µm

(wall persistent and adheres to spore in G41/6, Fig. 2.12). Aplanospores spherical to ellipsoidal (Fig. 2.15), 2-8 μm diameter, mostly with one to two chloroplasts, occasionally up to three. Zoospores 1.5-4.5 μm by 1-4 μm (5-8μm by 3-6.5 μm in 801 and WI/1, Fig. 2.14), spherical to pyriform to amoeboid (Fig. 2.13), with single chloroplast containing an anterior stigma; longer flagellum up to 8 μm long (up to 10 μm long in 801).

Vegetative division is frequent in both young and old cultures, (only constriction observed in G41/6). Vegetative division takes place in two ways. Firstly, spherical cells may become broadly ellipsoidal to almost cylindrical. A constriction usually appears in a median position (Figs. 3.13, 16), and the cell wall in this region often thickens and becomes faintly lamellate (Fig. 3.1). Thickenings are not always present (Figs. 3.7-9, 11, 15). The constriction becomes deeper (Figs. 3.2-4) and the cell wall thickenings closer together until the original protoplast is divided into two (Fig. 3.12). Before they separate, a thickish bridge of cell wall material may be present between the two daughter cells (Fig. 3.14). The daughter cells are sometimes of equal diameter and sometimes differ markedly (Figs. 3.8, 12). Secondly, division usually leads to two unequal daughter cells. The mother cell forms a bud which develops to form a daughter cell. Bud initiation commences with the formation of a thickening of one portion of the cell wall (Figs. 3.6, 10, 17). The mother cell becomes pyriform. The daughter bud then swells and becomes separated from the mother cell by a deepening constriction (Fig. 3.5). At the point of constriction, the cell wall is usually thickened. Eventually, cell wall material bridges the constriction and separates off the two protoplasts. These then separate as two daughter cells. A selection of cells exibiting various stages of division are shown in Figs. 3.7-17 and 4.4-5.

TEM. Chloroplast with parallel lamellae (Fig. 4.8), rarely interconnected between adjacent thylakoid bands. Pyrenoid absent (Fig. 4.7).

Distribution. Recorded from Cape Crozier, Scott Base, Victoria Valley, Granite Harbour and Windmill Islands.

Morphotype B2 (B. alpina Vischer)
(Figs. 5.1-19; 6.1-6 and 7.1-6)

LM. Cells spherical to ellipsoidal (Figs. 5.1-6 and 6.1, 5), up to 36 μm diameter, but mostly about 10 μm diameter, tending to form irregular aggregates (Figs. 5.3 and 6.5). Cell wall smooth, thin in young cultures but up to 4 μm thick, stratified, occasionally with one or several lens-shaped local thickenings up to 3.5 μm (Figs. 5.7 and 6.3) in old cultures, with superficial scattered deposition of small, amorphous iron oxide granules (Fig. 5.8). Chloroplasts more than 20 in adult cells, spindle to discoidal to polygonal, lacking pyrenoids (Figs. 5.1-6 and 6.1). Vacuoles large, scattered (Figs. 5.4, 9 and 6.2).

Sporangia up to 36 μm diameter (Figs. 5.10, 18-19), containing four to numerous (>16) spores (Fig. 5.19). Sporangium wall smooth, thin, occasionally up to 3 μm thick and stratified in old cultures. Spores released by rupture of sporangium wall (Fig. 5.11). Aplanospores spherical to ellipsoidal, 1.5-6.5 μm diameter, mostly with one to two chloroplasts, occasionally up to three (up to four chloroplasts in 897). Zoospores 1.25-5 μm by 1-3.5 μm, spherical, pyriform to amoeboid (Figs. 5.15-17), mostly with one, rarely two chloroplasts (Fig. 5.16) one of which has an anterior stigma; longer flagellum up to 5 μm long (up to 2 μm long in 895 and 897, Fig. 5.17). Vegetative division (Figs. 5.12-13) rare in young cultures but absent in old cultures.

TEM. Chloroplast with parallel lamellae interconnected between adjacent thylakoid bands (Fig. 7.2). Pyrenoid absent (Figs. 6.6 and 7.3).

SEM. Cell wall smooth in both young and old cultures (Figs. 7.5-6).

Distribution: Recorded from King George Island, Granite Harbour and Padda Island, Lützow Holm Bay.

Fig. 5. *Botrydiopsis* morphotype B2.

1-2, young cells. 3, loosely clustered cells. 4, two adult cells, one cell with a large vacuole (V). 5-6, spherical to ellipsoidal cells showing discoidal to polygonal chloroplasts which lack pyrenoids. 7, single cell with lens-shaped local wall thickening. 8, small, amorphous iron oxide granules scattered over the cell wall. 9, spherical cell with vacuoles. 10, zoosporangium with a thick, stratified wall and showing a distinct stigma in each chloroplast. 11, spores released by rupture of sporangium wall. 12-13, cells with constriction. 14, aplanospores with and without stigma. 15-17, zoospores. 15, zoospores each with a single chloroplast. 16, 17, pyriform and amoeboid zoospores each with one or two chloroplasts. 18-19, aplanosporangia. 19, an aplanosporangium with thick, stratified cell wall containing numerous aplanospores.

Scale bar is 10 μm.

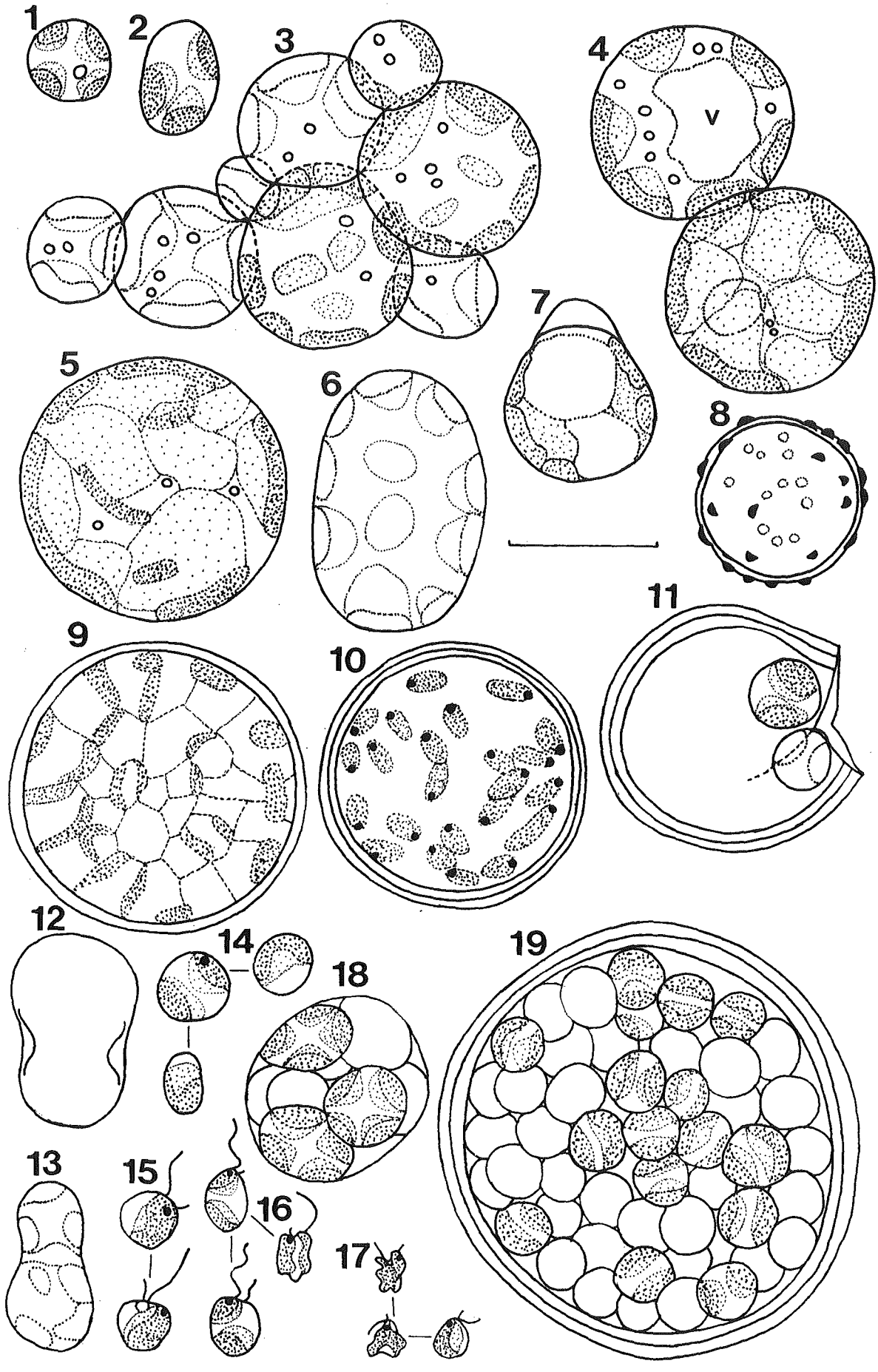
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Fig. 6. *Botrydiopsis* morphotype B2, LM (1-5) and TEM (6).

1, cell with chloroplasts lacking pyrenoids. 2, two cells, one with large vacuole (V). 3, cell with thick cell wall and lens-shaped thickening. 4, adult cells. 5, group of young cells. 6, cell in which the chloroplast clearly lacks a pyrenoid.

Scale in 1 also applies to 2-5.

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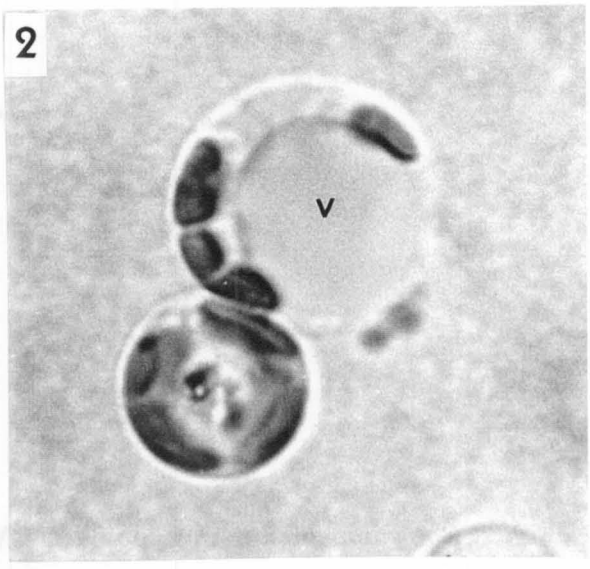
10μm

2



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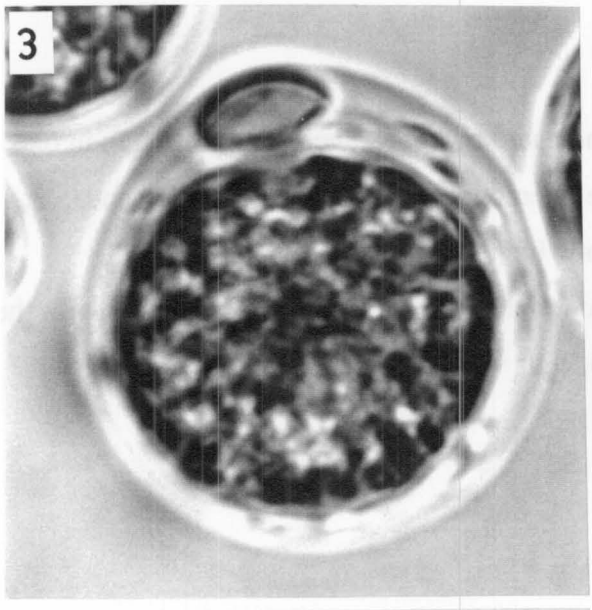

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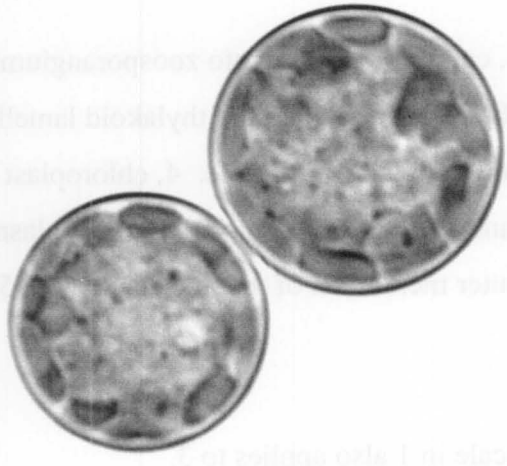

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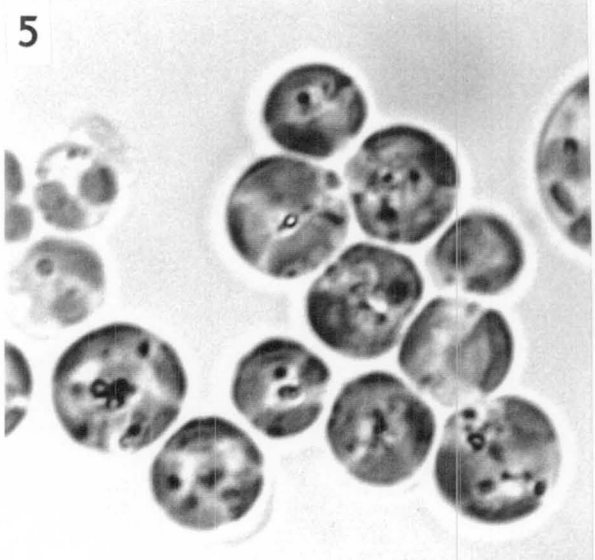


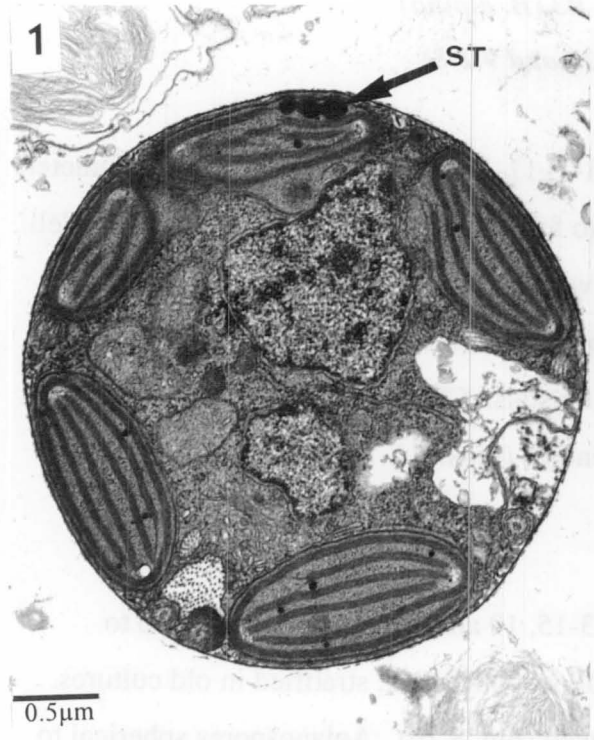
6

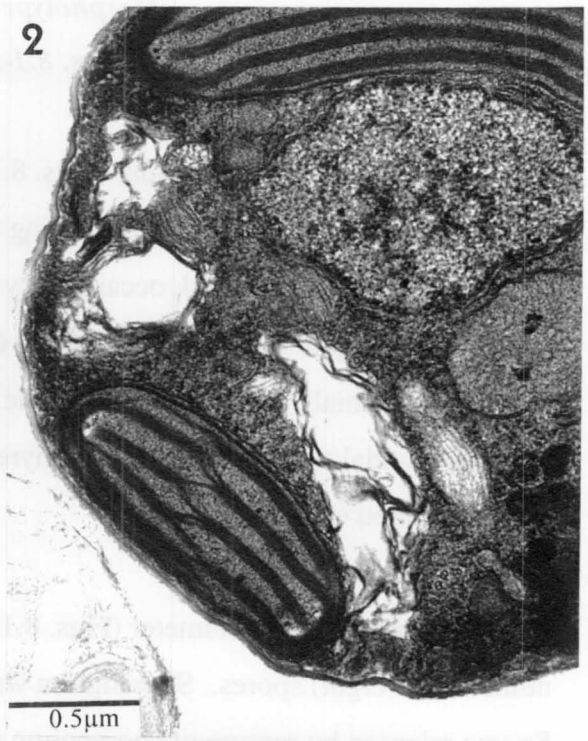
1μm

Fig. 7. *Botrydiopsis* morphotype B2, TEM (1-4) and SEM (5, 6).

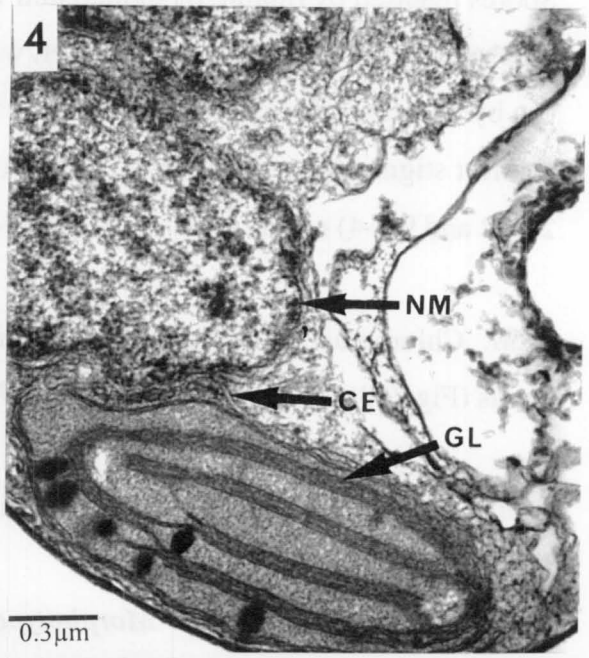
1, cell transforming into zoosporangium indicated by appearance of stigma (ST) in one chloroplast. 2, parallel, thylakoid lamellae interconnected between adjacent bands. 3, cell with a large vacuole. 4, chloroplast showing three-thylakoid lamellae, girdle lamellae (GL) and chloroplast endoplasmic reticulum (CE) which is continuous with the outer membrane of the nucleus (NM). 5-6, cells with smooth cell wall.

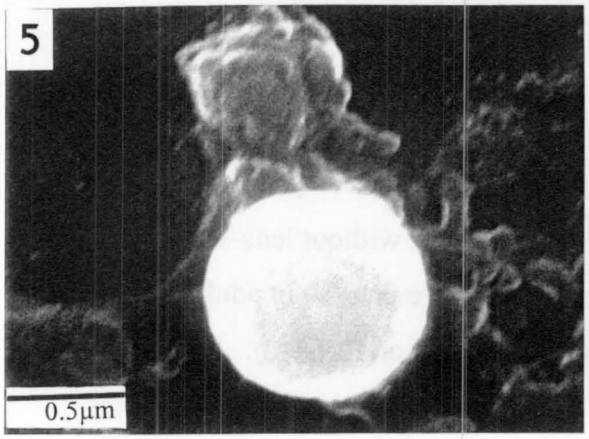
Scale in 1 also applies to 3.

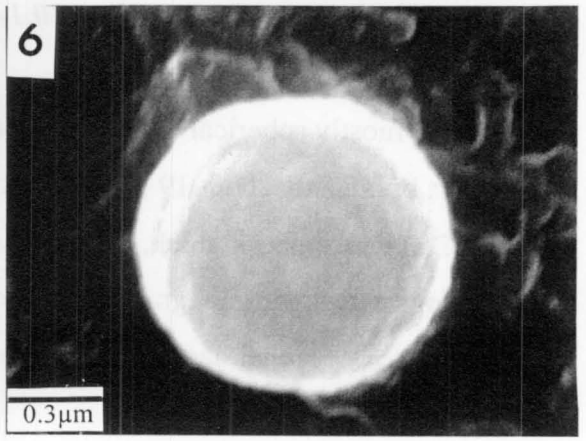

1
ST
0.5μm


2
0.5μm


3


4
NM
CE
GL
0.3μm


5
0.5μm


6
0.3μm

Morphotype B3 (B. alpina)
(Figs. 8.1-23 and 9.1-8)

LM. Cells spherical to ellipsoidal (Figs. 8.1-7, 11-12 and 9.1-2), up to 20 μm diameter, but mostly about 14 μm diameter, tending to form irregular aggregates (Fig. 8.3). Cell wall smooth, thick, stratified, occasionally with one or several lens-shaped local thickenings of up to 3 μm (Figs. 4.8-9) in old cultures, without superficial scattered deposition of small, amorphous iron oxide crystals. Chloroplasts more than 15 in adult cells, ellipsoidal to polygonal, lacking pyrenoids (Figs. 8.1-7 and 9.1-2). Vacuoles large, scattered (Fig. 4.10).

Sporangia up to 20 μm diameter (Figs. 8.13-15, 19 and 9.5-6), containing two to numerous (>eight) spores. Sporangium wall smooth, thick, stratified in old cultures. Spores released by rupture of sporangium wall (Fig. 8.16). Aplanospores spherical to ellipsoidal (Fig. 8.20), 2-8 μm diameter, with one to two chloroplasts. Zoospores 2.5-5 μm by 2-4 μm, spherical to pyriform (Fig. 8.23), with single chloroplast containing an anterior stigma; longer flagellum up to 5 μm long. Vegetative division (Figs. 8.17-18, 21-22 and 9.3-4) rare in both young and old cultures.

TEM. Chloroplast with parallel lamellae interconnected between adjacent thylakoid bands (Fig. 9.8). Pyrenoid absent (Fig. 9.7).

Distribution: Recorded from Chapman Ridge.

Morphotype B4 (B. alpina)
(Figs. 10.1-12 and 11.1-6)

LM. Cells mostly spherical to ellipsoidal (Figs. 10.1-5 and 11.1-2), up to 30 μm diameter, but mostly about 19 μm diameter, tending to form irregular aggregates (Fig. 11.6). Cell wall smooth, thick, stratified in old cultures, without lens-shaped local thickenings and superficial granules. Chloroplasts more than 20 in adult cells, ellipsoidal to polygonal, lacking pyrenoids (Figs. 10.1-5 and 11.1-2).

Sporangia up to 41.5 μm diameter (Figs. 10.7, 12), containing four to numerous (>16) spores (Figs. 11.3-4). Sporangium wall smooth, up to 2 μm thick, stratified in old cultures. Spores released by rupture of sporangium wall (Fig. 10.8). Aplanospores spherical to ellipsoidal (Fig. 10.10), 3-7 μm diameter, with one to two chloroplasts. Zoospores 4-5 μm by 2.5-4.5 μm, sub-spherical (Fig. 10.9), with one chloroplast containing an anterior stigma; longer flagellum up to 3 μm long. Vegetative division (Fig. 10.11) rare in young cultures but absent in old cultures.

TEM. Chloroplast with parallel lamellae interconnected between adjacent thylakoid bands (Fig. 11.6). Pyrenoid absent (Fig. 11.5).

Distribution: Recorded from Granite Harbour.

Morphotype B5 (B. arhiza)
(Figs. 12.1-19; 13.1-6 and 14.1-6)

LM. Cells mostly spherical to ellipsoidal (Figs. 12.1-5 and 13.1-2), single, up to 43.5 μm diameter, but mostly up to 17.5 μm diameter, not tending to aggregate. Cell wall up to 5 μm thick, stratified, occasionally with one or several lens-shaped local thickenings up to 3.5 μm (Figs. 12.8-9 and 13.3) in old cultures, without superficial granules; verrucose in occasional cells from young and old cultures (Figs. 12.6-7). Chloroplasts more than 20 in adult cells, ellipsoidal to polygonal, lacking pyrenoids (Figs. 12.1-5 and 13.1-2). Vacuoles large, scattered.

Sporangia up to 43.5 μm diameter (Figs. 12.10-13 and 13.4-5), containing four to numerous (>16) spores. Sporangium wall rarely verrucose, up to 5 μm thick, stratified in old cultures. Spores released by rupture of sporangium wall (Fig. 12.14). Aplanospores spherical to ellipsoidal (Fig. 12.16), 2-6.5 μm diameter, mostly with one or two chloroplasts, occasionally up to four. Zoospores 1-4 μm by 1-3 μm, sub-spherical to pyriform (Fig. 12.15), with two chloroplasts one of which has an anterior stigma; longer flagellum up to 4 μm long. Vegetative division (Figs. 12.17-19) rare in both young and old cultures.

Fig. 8. *Botrydiopsis* morphotype B3.

1-2, young cells. 3, loosely clustered cells. 4-6, spherical to ellipsoidal cells showing discoidal to polygonal chloroplasts which lack pyrenoids. 7, adult cell with thick, stratified cell wall. 8-9, single cell with lens-shaped local wall thickening. 10, cell with large vacuoles. 11-12, spherical adult cells. 13, zoosporangium with a thick, stratified wall, showing a distinct stigma in each chloroplast. 14, zoosporangium. 15, aplanosporangium with six aplanospores. 16, small sporangium and spores released by rupture of sporangium wall. 17, 21, young cells with constriction. 18, 22, commencement of "budding division". 19, sporangium with two spores. 20, aplanospores with and without stigma. 23, spherical to pyriform zoospores each with a single chloroplast containing an anterior stigma.

Scale bar is 10 μm.

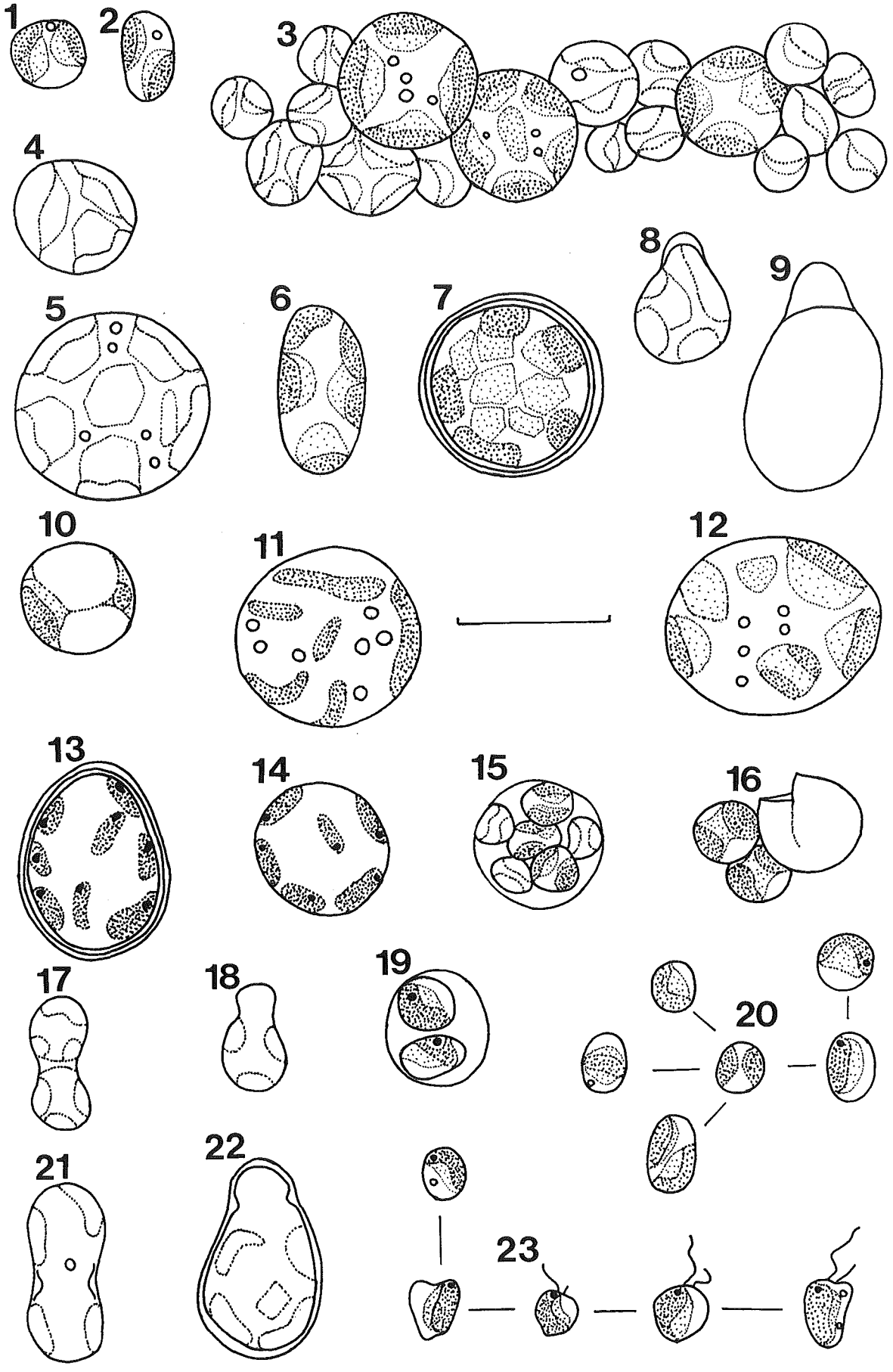

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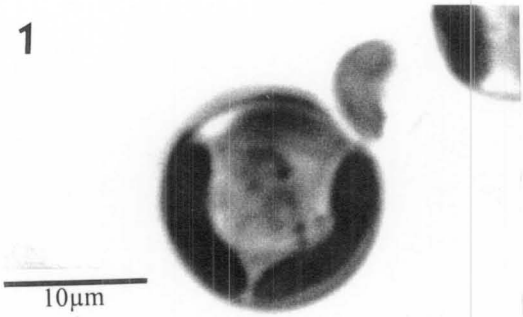
Fig. 9. *Botrydiopsis* morphotype B3, LM (1-6) and TEM (7, 8).

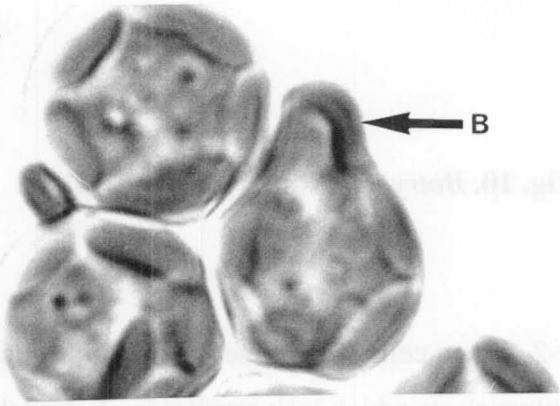
1, small spherical cell. 2, cells showing chloroplasts which lack pyrenoid. 3, early stage vegetative division. 4, group of cells, one cell showing "bud-like" projection (B). 5, aplanosporangium containing numerous aplanospores. 6, zoosporangium with zoospores and showing stigma (ST) in chloroplast. 7, cell in which the chloroplast lacks a pyrenoid. 8, chloroplast showing interconnection (I) between adjacent thylakoid bands.

Scale in 1 also applies to 2-6.

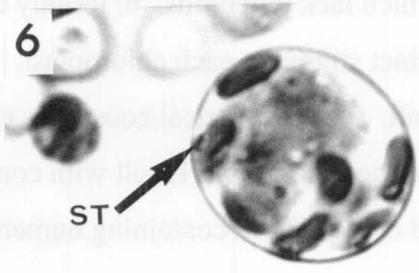

1
10μm


2


3


4
B


5


6
ST


7
1μm

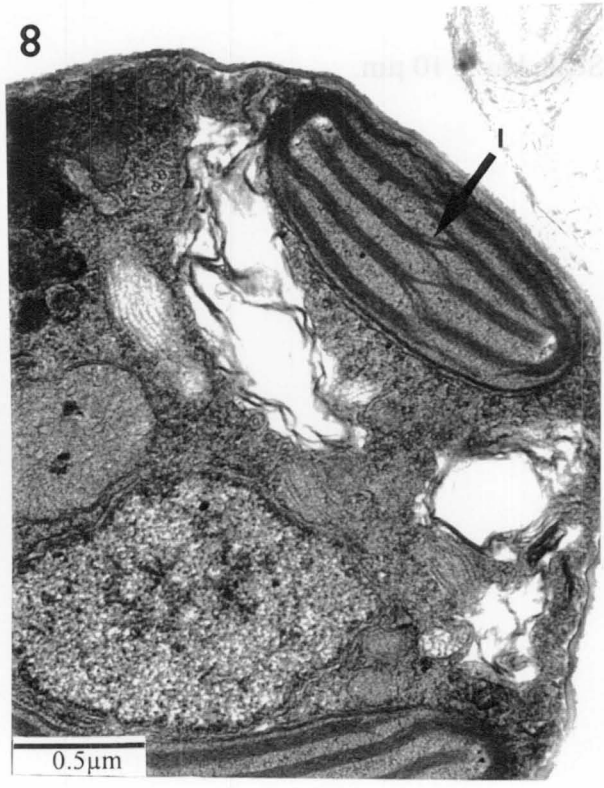

8
I
0.5μm

Fig. 10. *Botrydiopsis* morphotype B4.

1-2, young cells. 3-5, spherical to ellipsoidal cells showing ellipsoidal to polygonal chloroplasts which lack pyrenoids. 6, loosely clustered cells. 7, zoosporangium showing a distinct stigma in each chloroplast. 8, spores released by rupture of sporangium wall. 9, sub-spherical zoospores each with a single chloroplast. 10 aplanospores without stigma. 11, cell with constriction. 12, aplanosporangium with thick, stratified cell wall and containing numerous aplanospores.

Scale bar is 10 μm.

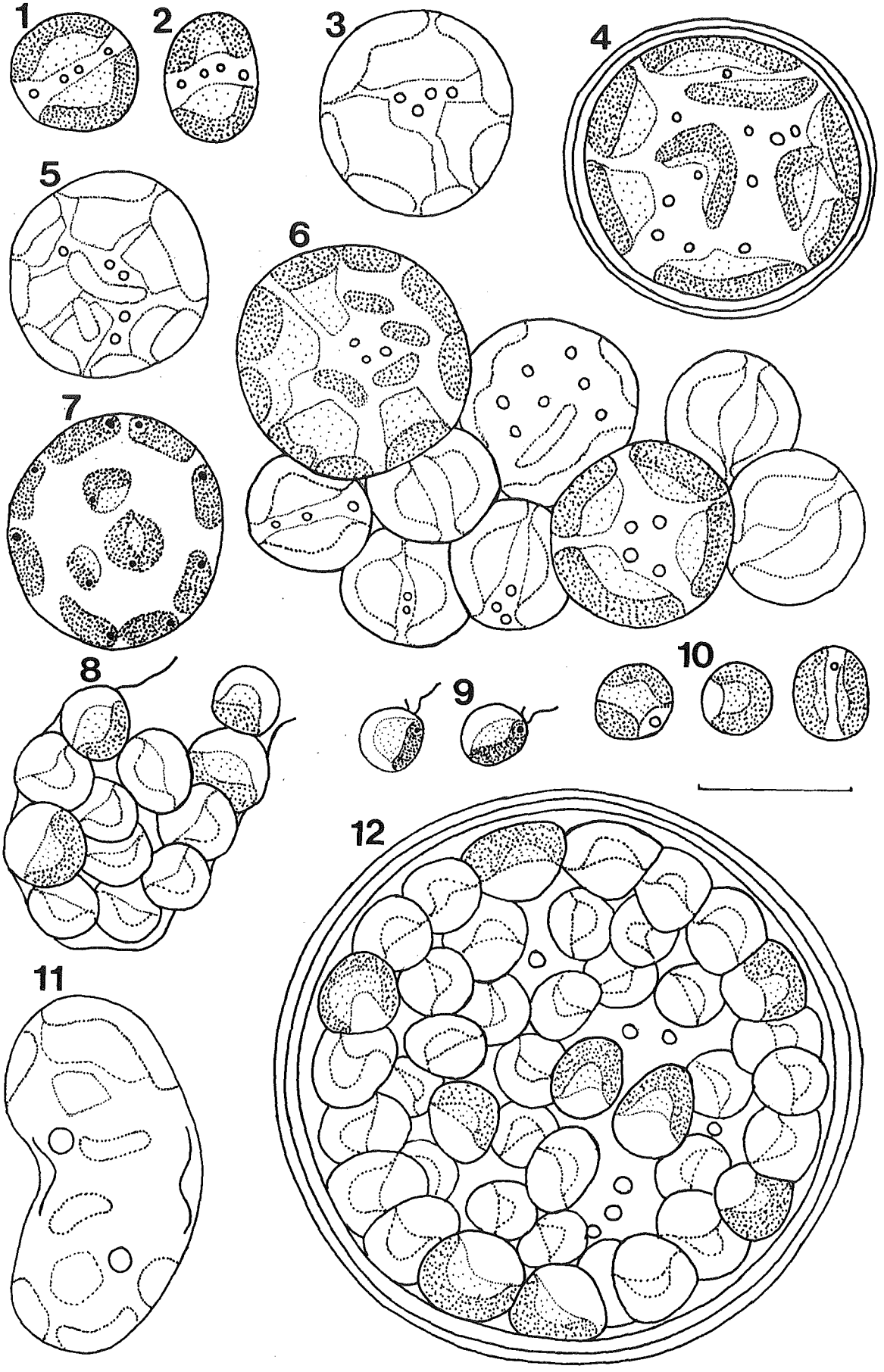

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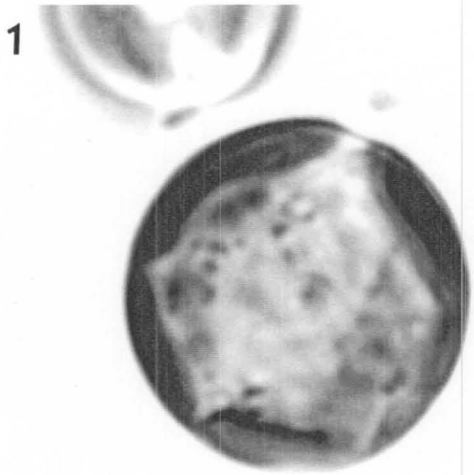
Fig. 11. *Botrydiopsis* morphotype B4, LM (1-4) and TEM (5, 6).

1, young cell. 2, adult cell. 3, 4, aplanosporangia containing numerous aplanospores. 5, vegetative cell with two chloroplasts which clearly lack pyrenoids; a nucleus (N) is associated with each chloroplast. 6, chloroplast showing parallel three-thylakoid lamellae interconnected (I) between adjacent thylakoid bands.

Scale in 1 also applies to 2-4.

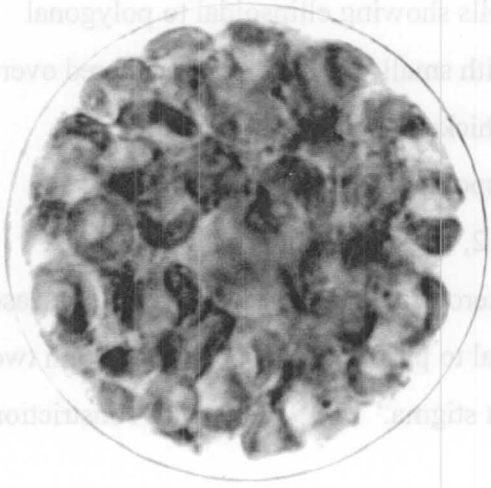
1



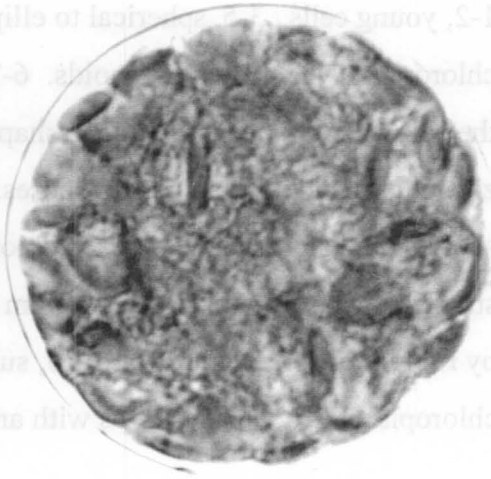
10μm

2

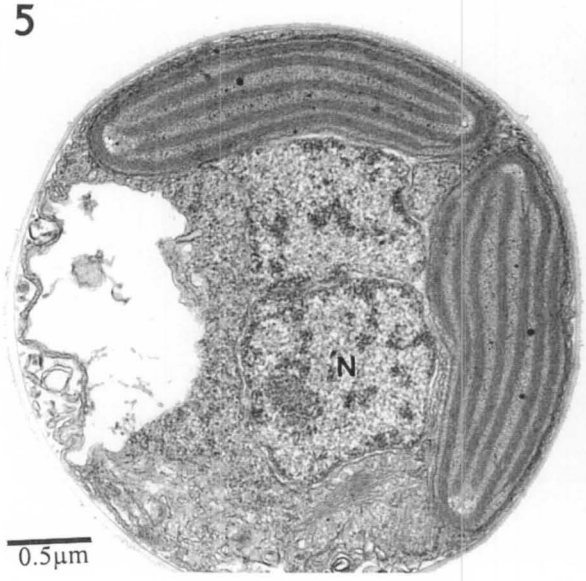
3



4

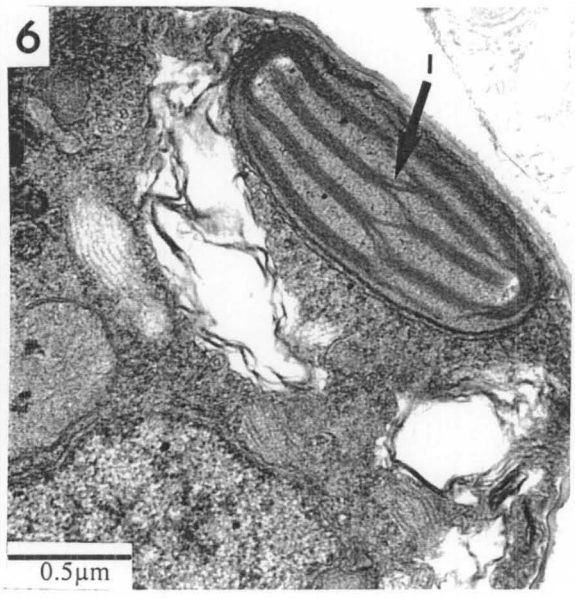


5



0.5μm

6



0.5μm

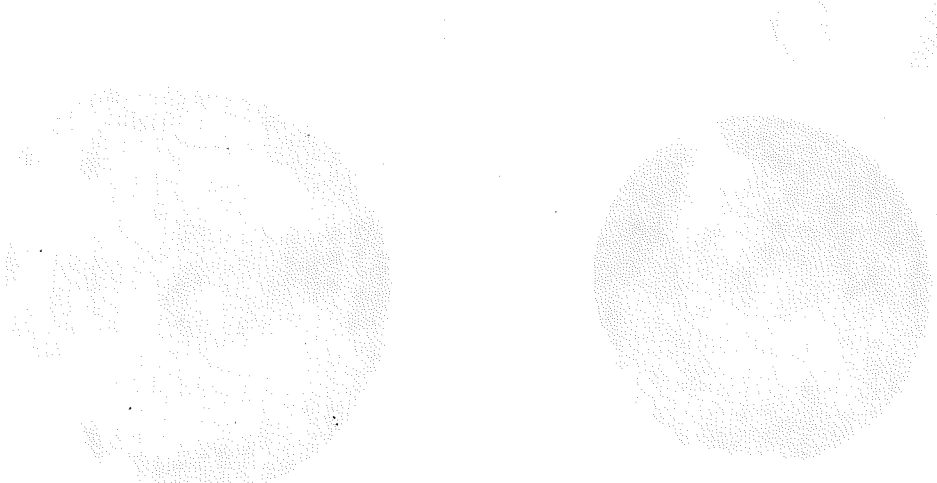


Fig. 12. *Botrydiopsis* morphotype B5.

1-2, young cells. 3-5, spherical to ellipsoidal cells showing ellipsoidal to polygonal chloroplasts which lack pyrenoids. 6-7, cells with small warts, densely scattered over the cell wall. 8-9, cells with lens-shaped local thickening. 10-13, sporangia. 10, zoosporangium with numerous spores. 11, zoosporangium with verrucose wall and showing a distinct stigma in each chloroplast. 12, zoosporangium with very thick, stratified wall. 13, aplanosporangium with numerous aplanospores. 14, spores released by rupture of sporangium wall. 15, sub-spherical to pyriform zoospores each with two chloroplasts. 16, aplanospores with and without stigma. 17-19, cells with constriction.

Scale bar is 10 μm.

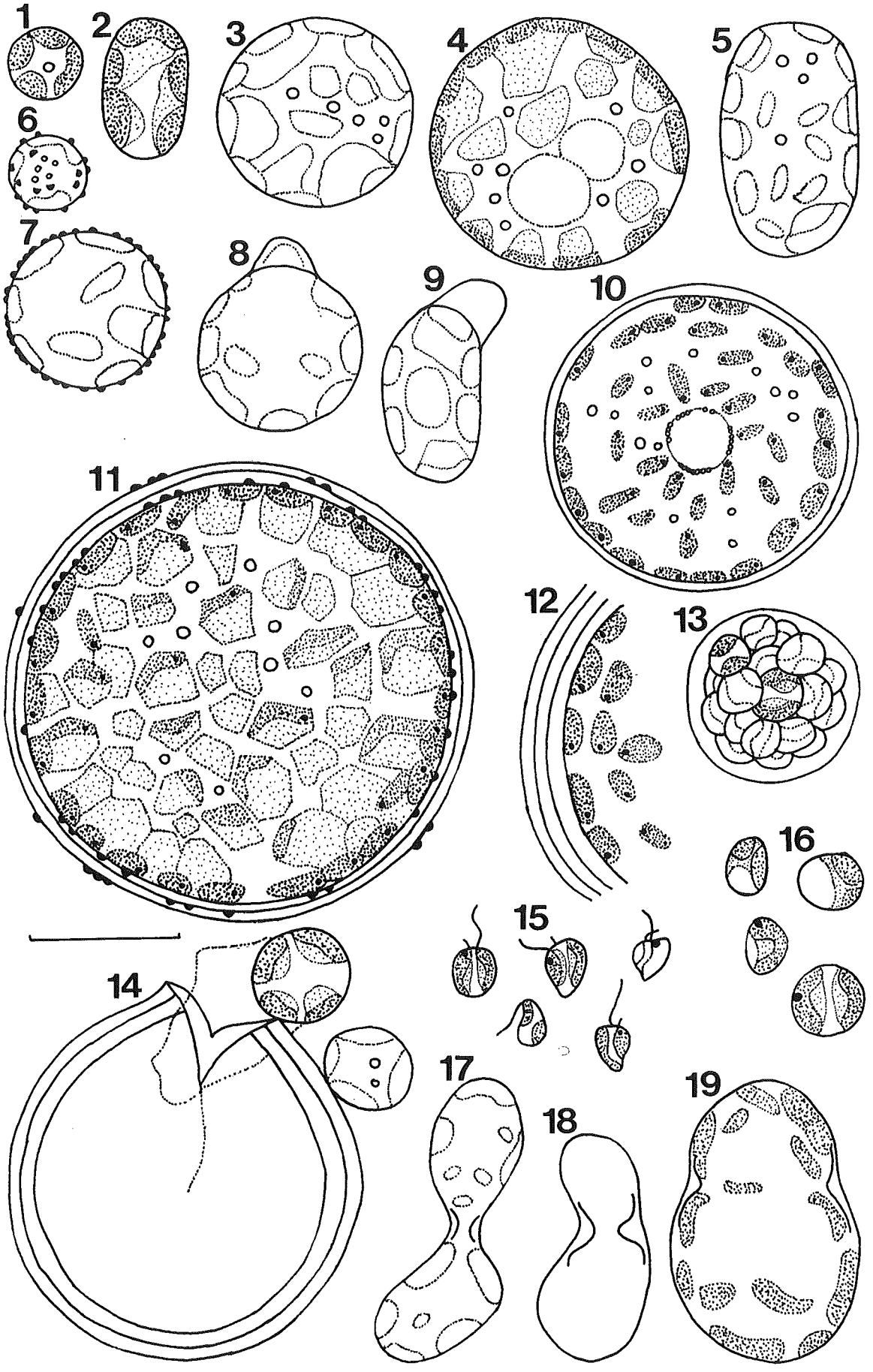

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Fig. 13. *Botrydiopsis* morphotype B5, LM (1-5) and TEM (6).

1, spherical cell showing chloroplasts which lack pyrenoids. 2, ellipsoidal cell. 3, cell with lens-shaped local wall thickening. 4, sporangia. 5, zoosporangium containing numerous zoospores and with thick wall. 6, cell with thick, verrucose wall.

Scale in 1 also applies to 2-5.

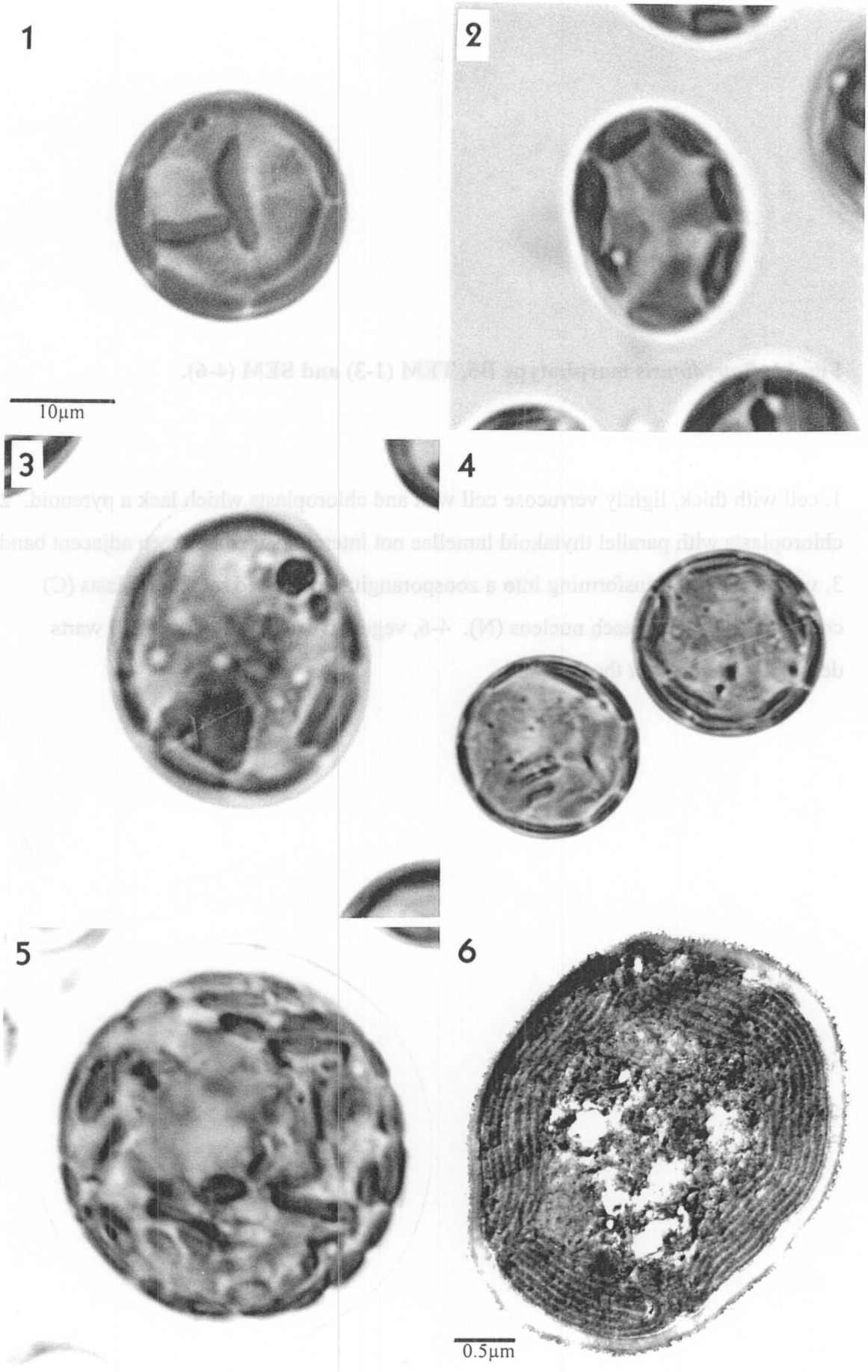
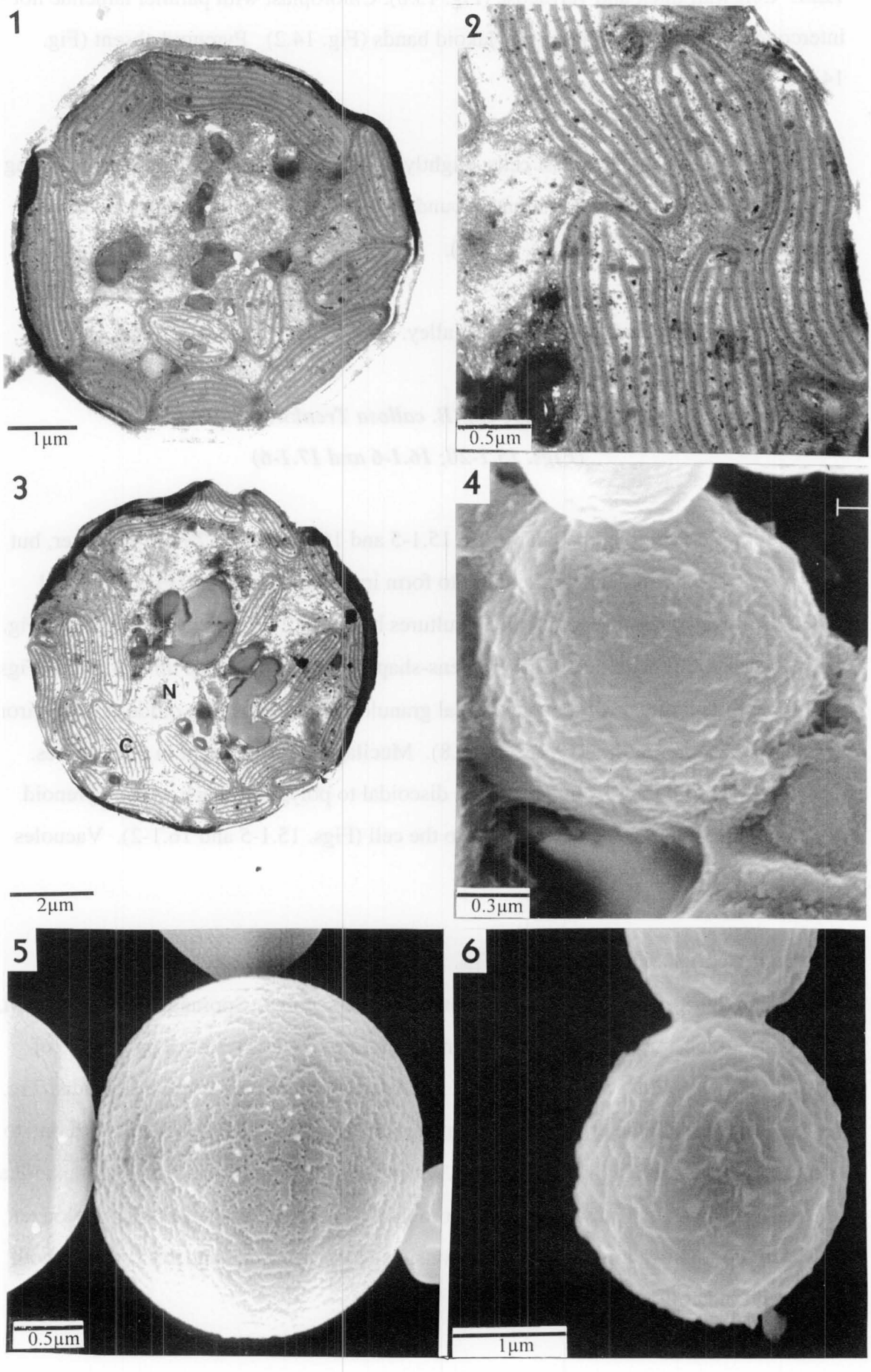

1
2
10µm
3
4
5
6
0.5µm

Fig. 14. *Botrydiopsis* morphotype B5, TEM (1-3) and SEM (4-6).

1, cell with thick, lightly verrucose cell wall and chloroplasts which lack a pyrenoid. 2, chloroplasts with parallel thylakoid lamellae not interconnected between adjacent bands. 3, vegetative cell transforming into a zoosporangium indicated by chloroplasts (C) coming to lie against each nucleus (N). 4-6, vegetative cells showing small warts densely scattered over the cell wall.


1
1μm
2
0.5μm
3
N
C
2μm
4
0.3μm
5
0.5μm
6
1μm

TEM. Cell wall thick and verrucose (Fig. 13.6). Chloroplast with parallel lamellae not interconnected between adjacent thylakoid bands (Fig. 14.2). Pyrenoid absent (Fig. 14.1).

SEM. Cell wall smooth in most cells, slightly verrucose in occasional cells from young and old cultures. Warts small, bluntly rounded, up to 0.65 μm in diameter, densely scattered over the cell wall (Figs. 14.4-6).

Distribution: Recorded from Victoria Valley.

Morphotype B6 (B. callosa Trenkwalder)
(Figs. 15.1-20; 16.1-6 and 17.1-6)

LM. Cells spherical to ellipsoidal (Figs. 15.1-5 and 16.1-2), up to 28 μm diameter, but mostly up to 11.5 μm diameter, tending to form irregular aggregates (Figs. 15.4 and 16.2). Cell wall smooth, thin in young cultures but up to 2 μm thick and stratified (Fig. 15.5), occasionally with one or several lens-shaped local thickenings up to 4.5 μm (Figs. 15.6-7) in old cultures, without superficial granules, amorphous iron oxide crystals (iron oxide granules present in G19/2, Fig. 15.8). Mucilage sheath present in old cultures. Chloroplasts more than 20 in adult cells, discoidal to polygonal, each with a pyrenoid which bulges from the surface facing into the cell (Figs. 15.1-5 and 16.1-2). Vacuoles large, scattered (Fig. 15.10).

Sporangia up to 30.5 μm diameter (Figs. 15.9, 11-12, 14 and 16.4, 6) (up to 35 μm diameter in G9/8), containing two to numerous (>16) spores. Sporangium wall smooth, up to 2 μm thick, stratified (Fig. 15.16) in old culture. Spores released by rupture of sporangium wall (Figs. 15.13, 15 and 16.3). Aplanospores spherical to ellipsoidal (Fig. 15.18), 3.5-8 μm diameter (12.5 μm diameter in G99/2, Fig. 15.14), mostly with one to two chloroplasts, occasionally up to four. Zoospores 2.5-5 μm by 2-5 μm, sub-spherical to pyriform (Figs. 15.17), with single chloroplast containing an anterior stigma; longer flagellum up to 5 μm long. Vegetative division (Figs. 15.19-20 and 16.5) rare in both young and old cultures.

TEM. Chloroplast with lamellae interconnected between adjacent thylakoid bands, but only in matrix of the pyrenoid (Fig. 17.4). Thylakoid lamellae more widely spaced in pyrenoid than in remainder of chloroplast (Fig. 17.6). Pyrenoid projecting from chloroplast as a rounded bulge (Figs. 17.1-3), traversed by thylakoid lamellae (Fig. 17.4).

Distribution: Recorded from Victoria Valley, Granite Harbour and Castle Rock.

Morphotype B7 (B. callosa)
(Figs. 18.1-15; 19.1-6 and 20.1-5)

LM. Cells spherical (Figs. 18.1-5 and 19.1-2), up to 31 μm diameter, but mostly up to 19 μm diameter, single or tending to form irregular aggregates (Fig. 18.7). Cell wall smooth, thin in young cultures but up to 4 μm thick, stratified, occasionally with one or several lens-shaped local thickenings up to 3.5 μm (Figs. 18.11 and 19.3) in old cultures, and with superficial scattered deposition of small, amorphous iron oxide granules (Fig. 18.6). Chloroplasts more than 20 in adult cells, spindle to discoid. Pyrenoid either indistinct, not projecting from chloroplast or distinct bulging from chloroplast (Figs. 18.1-5 and 19.1-2). Vacuoles large, scattered (Figs. 18.5, 8).

Sporangia up to 31 μm diameter (Figs. 18.9-10, 16 and 19.4-6), containing four to numerous (>20) spores. Sporangium wall smooth, thin, occasionally up to 4 μm thick, stratified in old cultures. Spores released by rupture of sporangium wall (Fig. 18.12). Aplanospores spherical (Fig. 18.15), 1.5-12.5 μm diameter, mostly with one to two chloroplasts, occasionally up to seven. Zoospores 1.25-5 μm by 1-4.5 μm, sub-spherical to pyriform (Fig. 18.14), with a single chloroplast containing an anterior stigma; longer flagellum up to 7.5 μm long. Vegetative division (Fig. 18.13) rare in both young and old cultures.

TEM. In strain 877, chloroplast with parallel lamellae frequently interconnected between adjacent thylakoid bands (Figs. 20.4). Pyrenoid lying below convex inner face of chloroplast, immersed, traversed by more widely spaced parallel lamellae than in the

Fig. 15. *Botrydiopsis* morphotype B6.

1-2, young cells. 3-5, spherical to ellipsoidal cells showing discoidal to polygonal chloroplasts with pyrenoids projecting towards interior of cell. 5, adult cell with stratified wall. 6-7, cells with lens-shaped local wall thickening. 8, small, amorphous iron oxide granules scattered over the cell wall. 9, zoosporangium showing a distinct stigma in each chloroplast. 10, spherical cell with large vacuoles. 11, sporangium with thin mucilage sheath. 12, aplanosporangium with numerous aplanospores. 13, 15, spores released by rupture of sporangium wall. 14, in strain G99/7, aplanosporangium with two, large aplanospores each with four chloroplasts. 16, zoosporangium with thick, stratified wall. 17, sub-spherical to pyriform zoospores each with a single chloroplast containing an anterior stigma. 18, aplanospores with and without stigma. 19-20, cells with constriction undergoing vegetative division.

Scale bar is 10 μm.

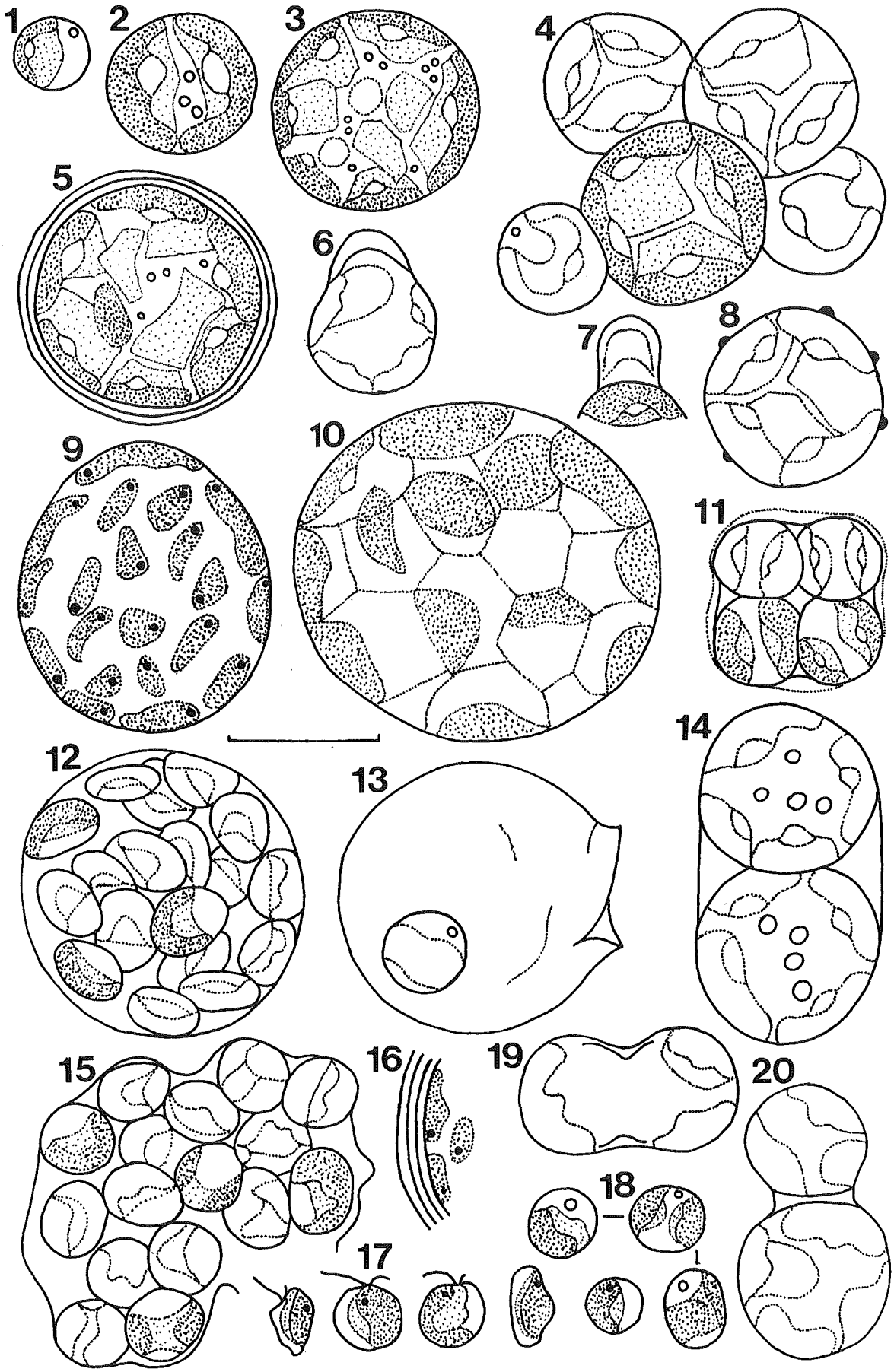

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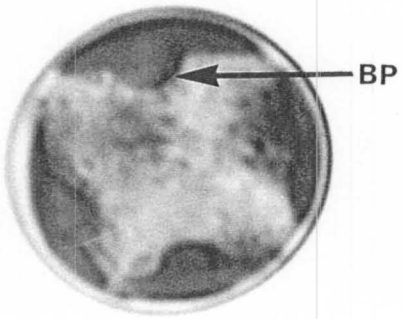
Fig. 16. *Botrydiopsis* morphotype B6, LM.

1, cell showing pyrenoid projecting from chloroplast as a rounded bulge (BP). 2, group of young cells each with a distinct bulged pyrenoid (BP) projecting from each chloroplast. 3, vegetative cell and ruptured aplanosporangium with two aplanospores. 4, 6, sporangia. 5, cell at early stage of vegetative division.

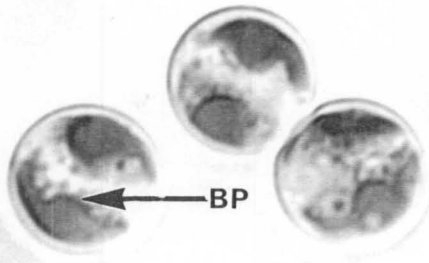
Scale in 1 also applies to 2-6.

1

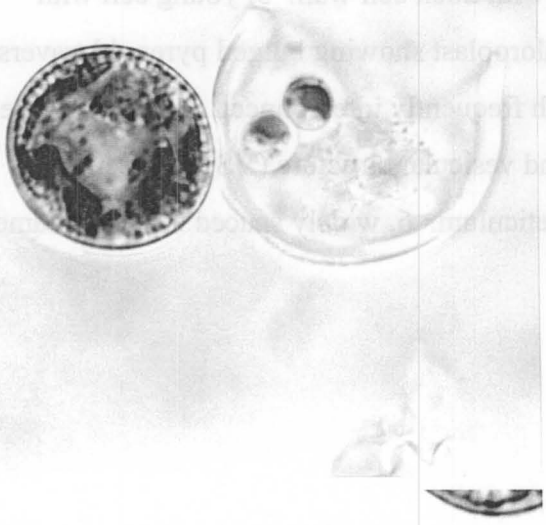


10µm

2



3



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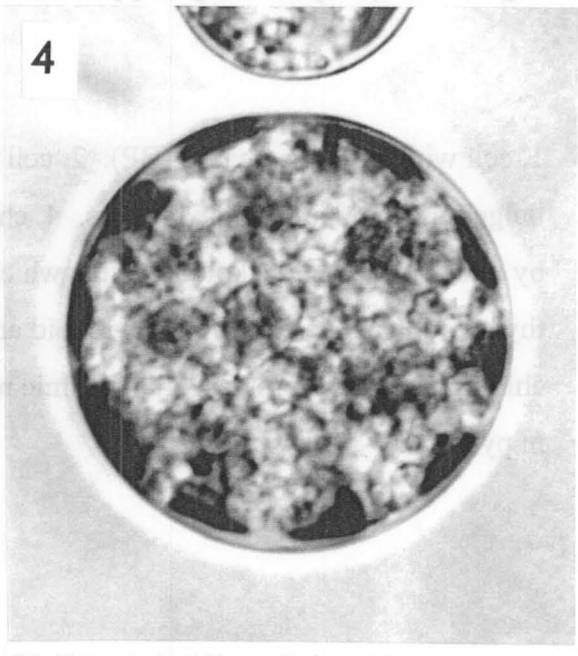

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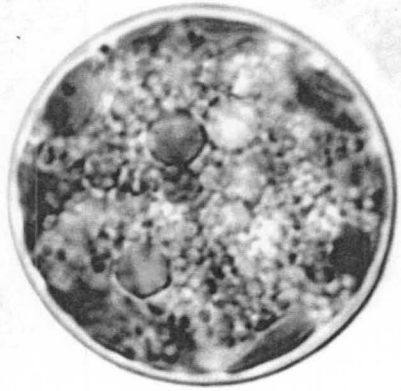
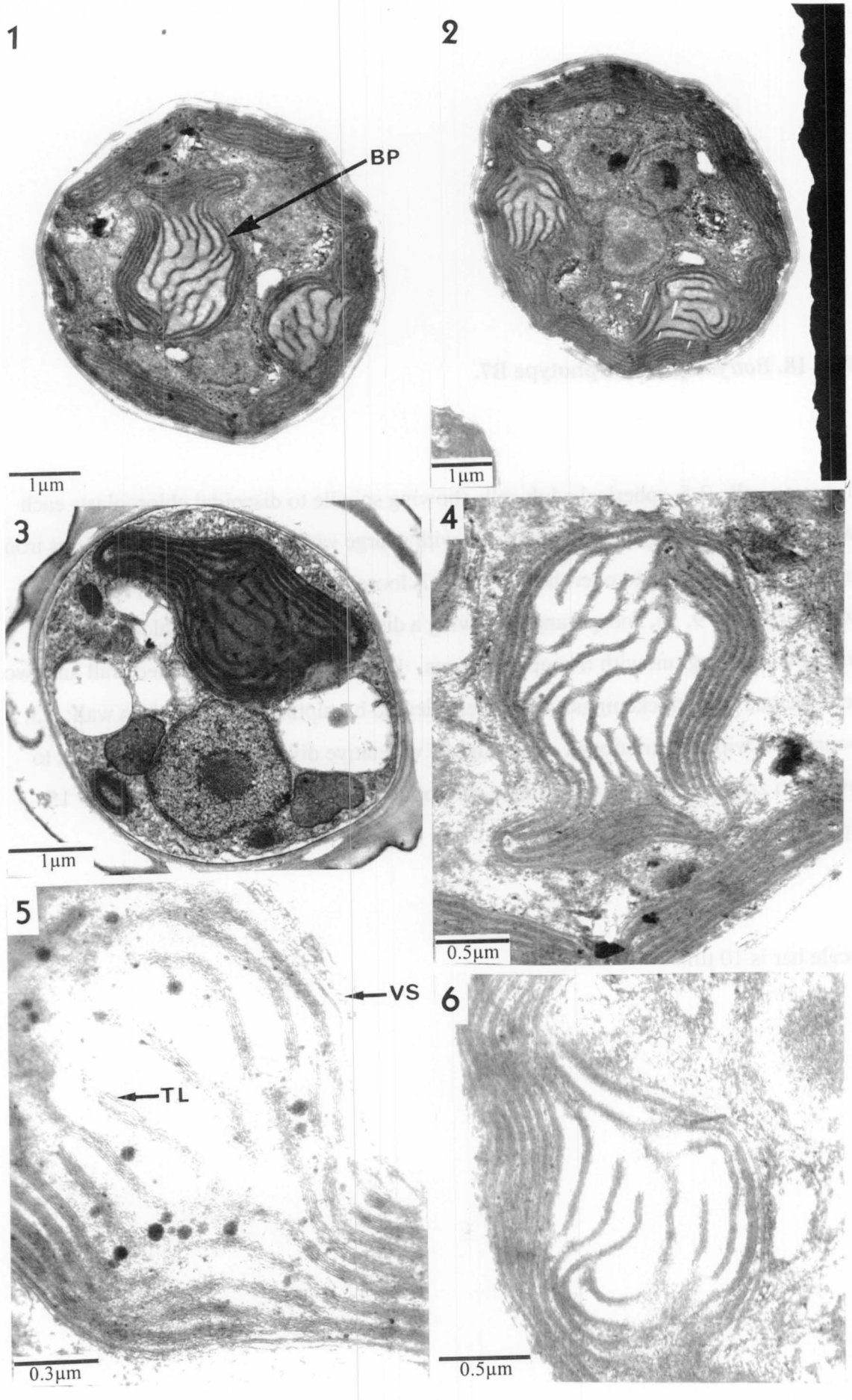


Fig. 17. *Botrydiopsis* morphotype B6, TEM.

1, cell with bulged pyrenoid (BP). 2, cell with thick cell wall. 3, young cell with bulged pyrenoid and single nucleus. 4, chloroplast showing bulged pyrenoid traversed by widely spaced thylakoid lamellae which frequently interconnect. 5, detail of three-thylakoid lamellae (TL) in the pyrenoid and vesicular structure (VS) between chloroplast and chloroplast endoplasmic reticulum. 6, widely spaced thylakoid lamellae in pyrenoid.


1
BP
1μm
2
1μm
3
1μm
4
0.5μm
5
VS
TL
0.3μm
6
0.5μm

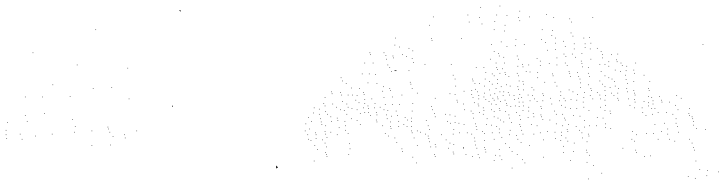


Fig. 18. *Botrydiopsis* morphotype B7.

1, young cell. 2-5, spherical adult cells showing spindle to discoidal chloroplasts each with an indistinct pyrenoid. 5, adult cell with a large vacuole. 6, small, amorphous iron oxide granules scattered over the cell wall. 7, loosely clustered cells. 8, spherical cell with vacuoles. 9, 16, zoosporangia showing a distinct stigma in each chloroplast. 10, an aplanosporangium with five aplanospores. 11, cell with thick, stratified wall and two lens-shaped local thickenings. 12, spores released by rupture of sporangium wall. 13, young cell with constriction at early stage of vegetative division. 14, sub-spherical to pyriform zoospores each with a single chloroplast containing an anterior stigma. 15, aplanospores with and without stigma.

Scale bar is 10 μm.

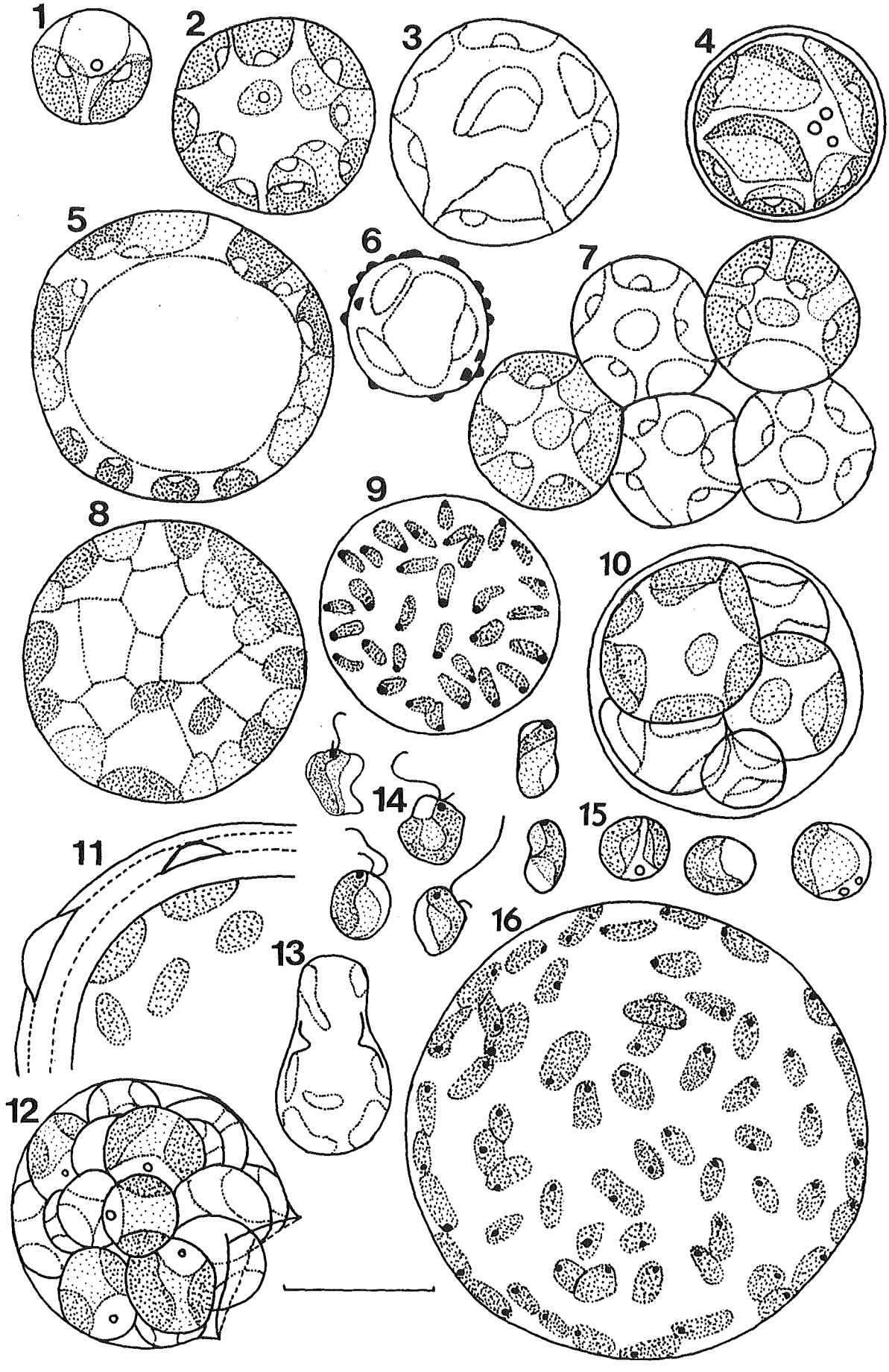


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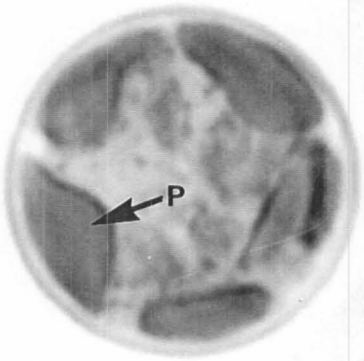
Fig. 19. *Botrydiopsis* morphotype B7, LM.

1, cell with indistinct pyrenoid (P). 2, two vegetative cells, one with distinct bulged pyrenoid (BP). 3, cell with lens-shaped wall thicking. 4, sporangium with large vacuoles. 5, sporangium with thick wall. 6, group of sporangia each with a stratified wall.

Scale in 1 also applies to 2-6.

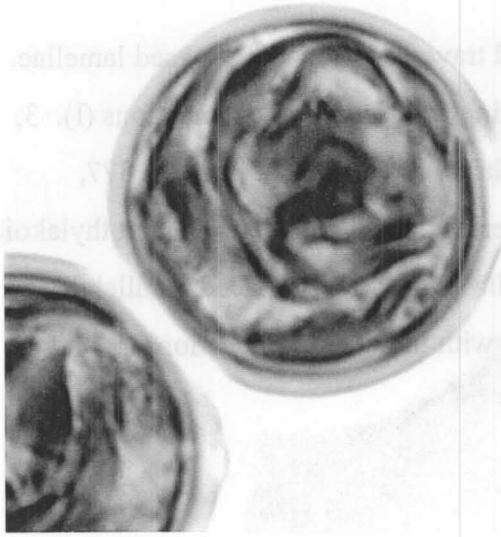
1



10µm

2

3



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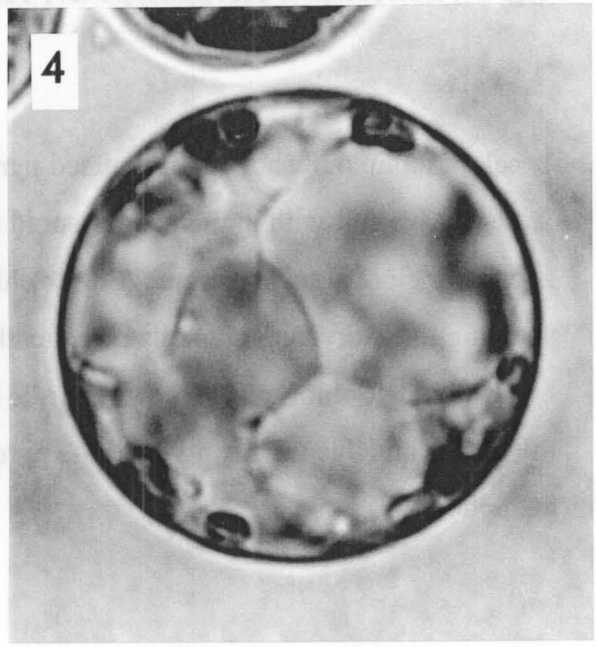


5

6

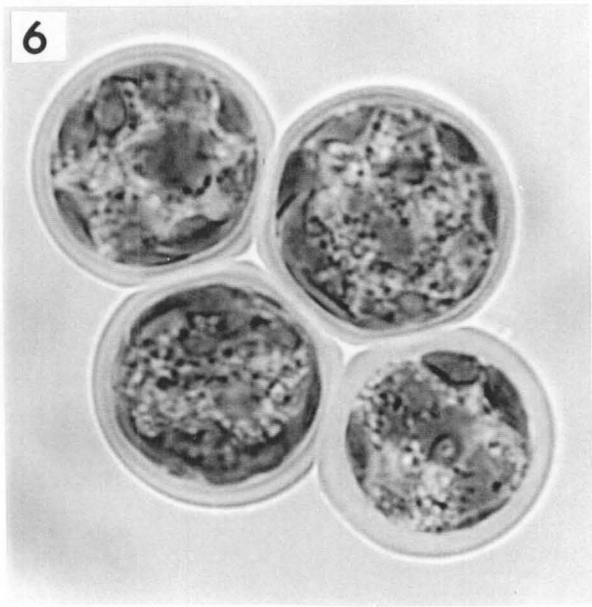
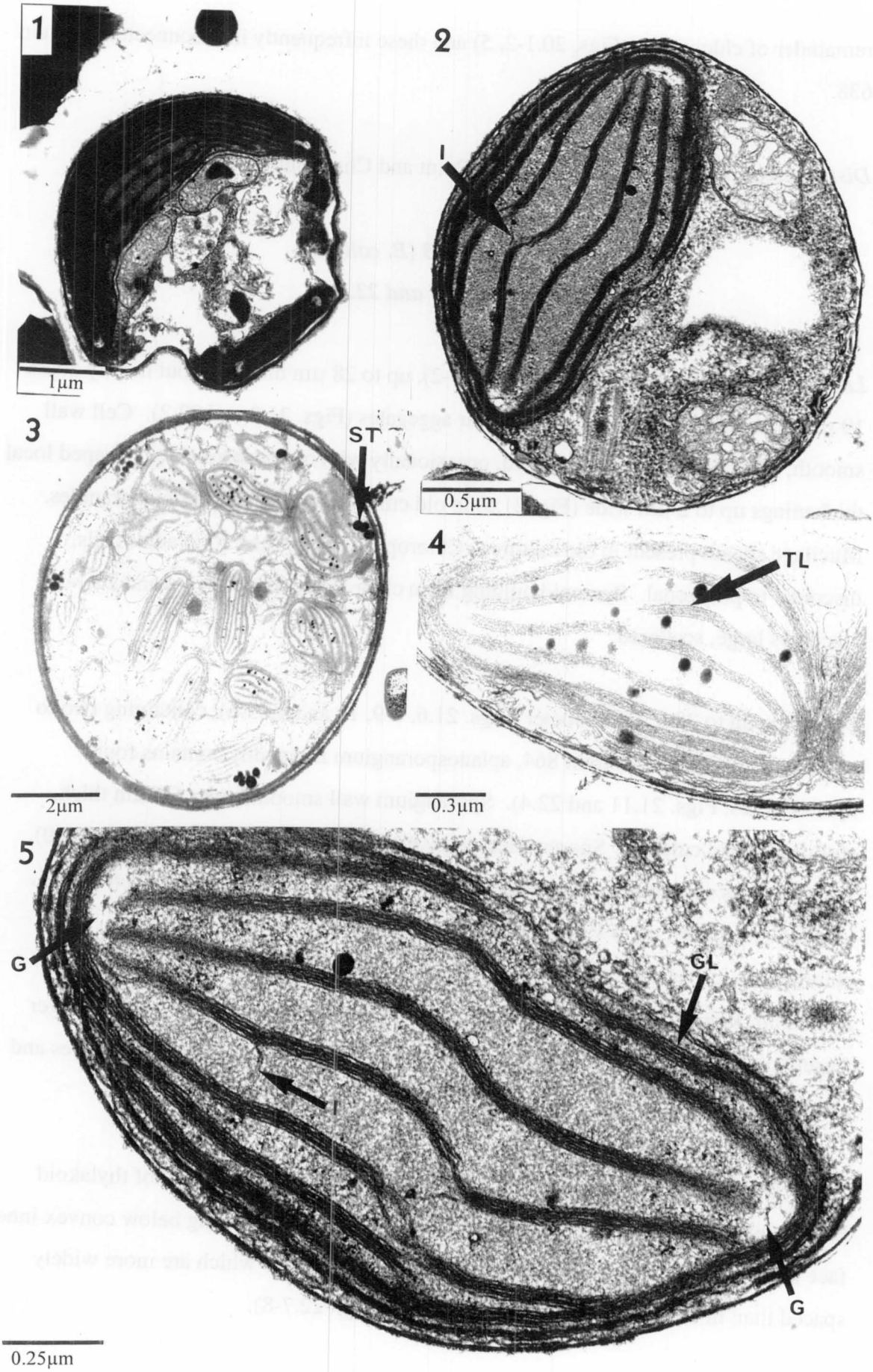


Fig. 20. *Botrydiopsis* morphotype B7, TEM.

1, cell of strain 638 showing immersed pyrenoid traversed by widely spaced lamellae. 2, vegetative cell with immersed pyrenoid showing few, indistinct connections (I). 3, zoosporangium showing stigma (ST) in chloroplast. 4, chloroplast in strain 877, showing three-thylakoid lamellae frequently interconnected between adjacent thylakoid bands (TL). 5, chloroplast showing girdle lamella (GL), widely spaced parallel three-thylakoid lamellae traversing the large pyrenoid with indistinct connections (I) and genophore (G).


1
1µm
2
I
0.5µm
3
ST
2µm
4
TL
0.3µm
5
G
GL
I
G
0.25µm

remainder of chloroplast (Figs. 20.1-2, 5) and these infrequently interconnected in strain 638.

Distribution: Recorded from Edmonson Point and Christchurch.

Morphotype B8 (B. callosa)
(Figs. 21.1-14 and 22.1-8)

LM. Cells spherical (Figs. 21.1-4 and 22.1-2), up to 28 μm diameter, but mostly about 19 μm diameter, tending to form irregular aggegates (Figs. 21.3 and 22.2). Cell wall smooth, up to 6.5 μm thick, stratified, occasionally with one or several lens-shaped local thickenings up to 2 μm wide (Fig. 21.5) in old cultures, without superficial granules. Mucilage sheath present in old cultures. Chloroplasts more than 20 in adult cells, discoidal to polygonal. Pyrenoid bulging from chloroplast (Figs. 21.1-4 and 22.1-2). Vacuoles large, scattered.

Sporangia up to 28.5 μm diameter (Figs. 21.6, 8-9, 11 and 22.3-6), containing two to numerous (>16) spores (strain 864, aplanosporangium frequently contains four aplanospores, Figs. 21.11 and 22.4). Sporangium wall smooth, up to 6.5 μm thick, stratified in old cultures. Spores released by rupture of sporangium wall (Fig. 21.10). Aplanospores spherical to ellpsoidal (Figs. 21.14 and 22.3-4), 2-7 μm diameter (864, large aplanospores, up to 19 μm diameter, Fig. 21.9), mostly with up to five chloroplasts, occasionally up to 14. Zoospores 1.25-5.5 μm by 1-3 μm, pyriform to amoeboid (Figs. 21.13), with single chloroplast containing an anterior stigma; longer flagellum up to 3 μm long. Vegetative division (Fig. 21.12) rare in young cultures and absent in old cultures.

TEM. Chloroplast with parallel thylakoid lamellae branched at the end of thylakoid bands, only those traversing pyrenoid (Figs. 22.7-8). Pyrenoid lying below convex inner face of chloroplast, immersed, traversed by parallel lamellae which are more widely spaced than in the remainder of the chloroplast (Figs. 22.7-8).

Distribution: Recorded from Granite Harbour and Christchurch.

Morphotype B9 (B. callosa)
(Figs. 23.1-14 and 24.1-8)

LM. Cells spherical (Figs. 23.1-5 and 24.1-3), single, up to 22.5 μm diameter, but mostly about 15 μm diameter. Cell wall smooth, thin in young cultures but up to 4 μm thick (Figs. 23.12 and 24.4), stratified, occasionally with one or several lens-shaped local thickenings up to 3 μm wide (Figs. 23.9, 13) in old cultures, without superficial granules. Chloroplasts more than 20 in adult cells, spindle-shaped. Pyrenoid bulging from inner face of chloroplast (Figs. 23.1-5 and 24.1-3). Vacuoles large, scattered (Fig. 23.6).

Sporangia up to 22 μm diameter (Figs. 23.7-8 and 24.5-6), containing four to numerous (>16) spores. Sporangium wall smooth, up to 4 μm thick, stratified in old cultures. Spores released by rupture of sporangium wall (Fig. 23.14). Aplanospores spherical (Fig. 23.11), 2.5-4.9 μm diameter, mostly with one chloroplast, occasionally two. Zoospores 2.5-4.5 μm by 1.5-4.5 μm, sub-spherical to pyriform (Figs. 23.10), with single chloroplast containing an anterior stigma; longer flagellum up to 7.5 μm long. Vegetative division absent in both young and old cultures.

TEM. Moderately fixed chloroplast with thylakoid lamellae passing through pyrenoid. Pyrenoid bulged (Figs. 24.7-8).

Distribution: Recorded from Casey Station area.

***Botryochloris* Pascher 1930**

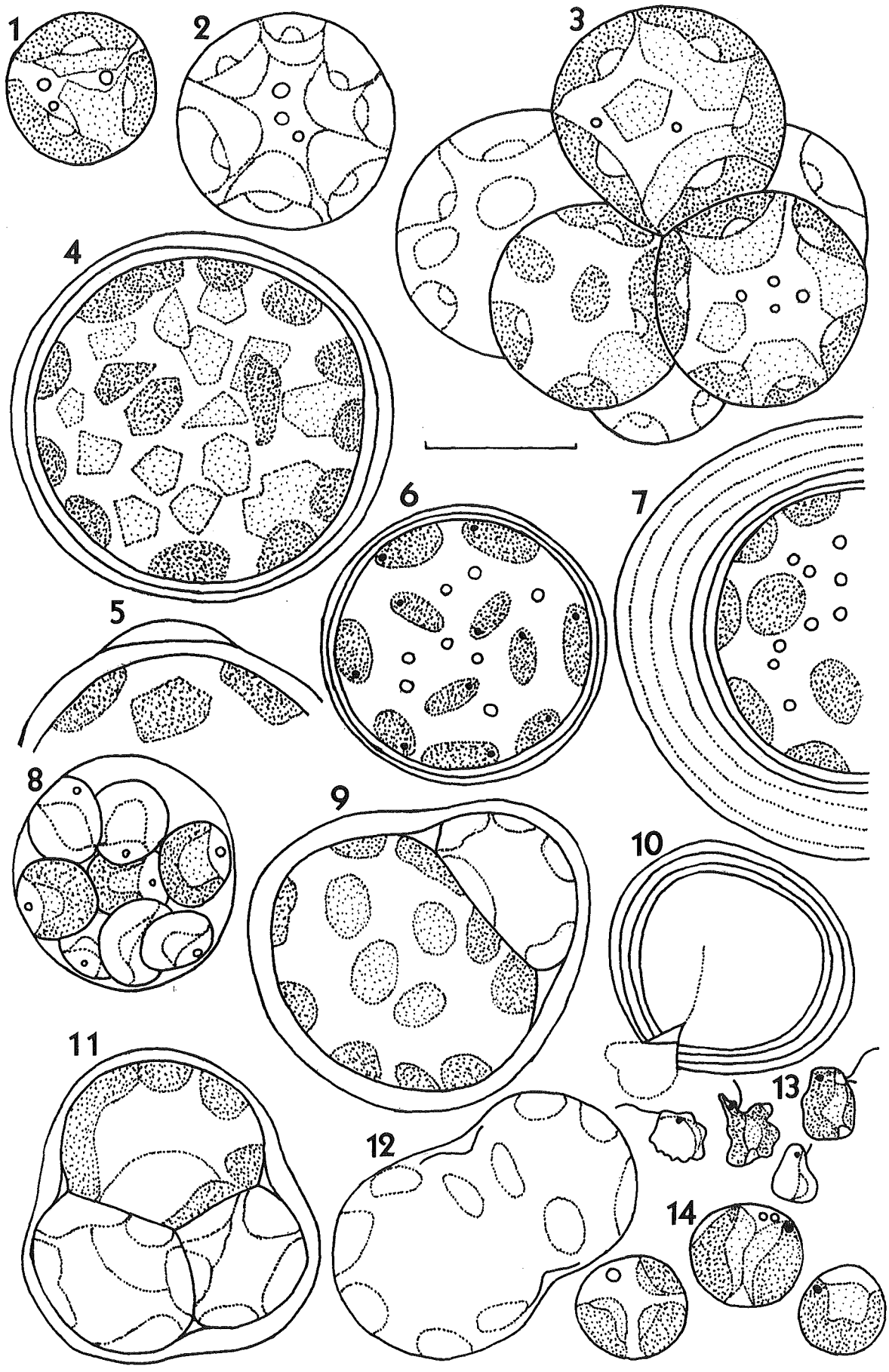
Botryochloris was established by Pascher (1930) with the following diagnosis (translated from the German):

"Cells form small, grape-shaped colonies with 100 or more cells which easily separate into smaller colonies. Cells are densely packed, sometimes slightly flattened, otherwise

Fig. 21. *Botrydiopsis* morphotype B8.

1, young cell. 2-4, spherical cells showing discoidal to polygonal chloroplasts each with a pyrenoid. 5, cell with lens-shaped local thickening. 6, zoosporangium with a thick, stratified wall and showing a distinct stigma in each chloroplast. 7, adult cell with very thick, stratified wall. 8, aplanosporangium with eight aplanospores. 9, strain 864, aplanosporangium with two large aplanospores with numerous chloroplasts. 10, spores released by rupture of sporangium wall. 11, aplanosporangium with four aplanospores frequently found in strain 864. 12, cell with constriction. 13, pyriform to amoeboid zoospores each with a single chloroplast containing an apical stigma. 14, aplanospores with and without stigma.

Scale bar is 10 μm.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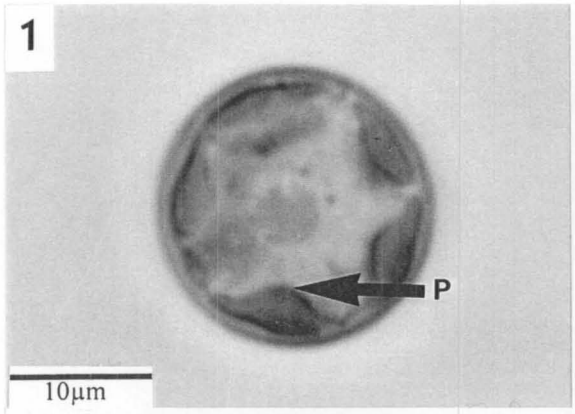
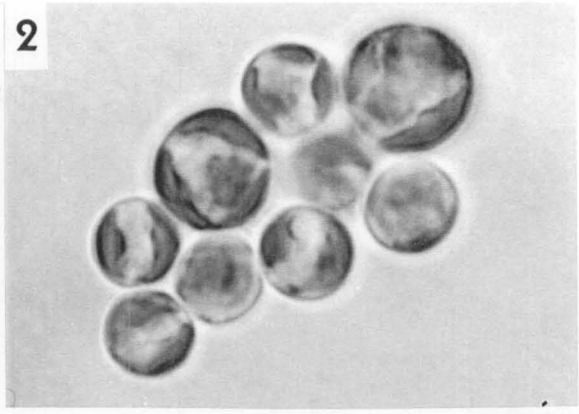
13
14

Fig. 22. *Botrydiopsis* morophotype B8, LM (1-6) and TEM (7, 8).

1, cell with a pyrenoid (P). 2, group of young cells. 3, aplanosporangium with two aplanospores. 4, aplanosporangium with four aplanospores. 5, zoosporangium with thick wall. 6, aplanosporangium with eight aplanospores. 7, cell with an immersed pyrenoid in each chloroplast; thylakoid lamellae interconnected (I) at the end of thylakoid bands. 8, chloroplast showing pyrenoid traversed by widely spaced thylakoids.

Scale in 1 also applies to 2-6.


1
P
10μm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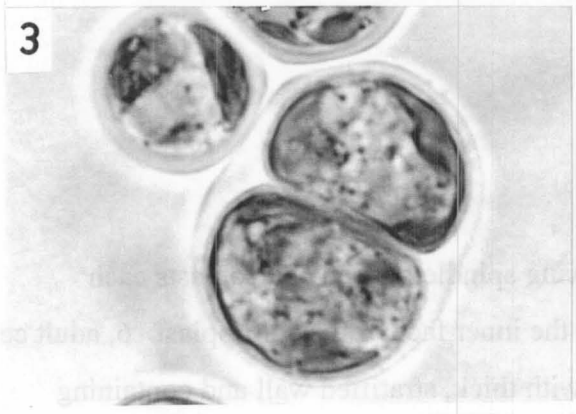
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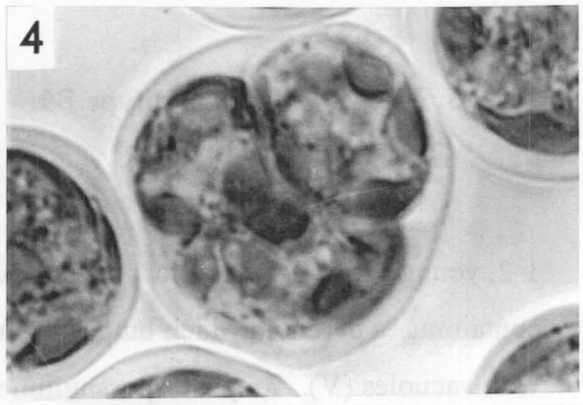
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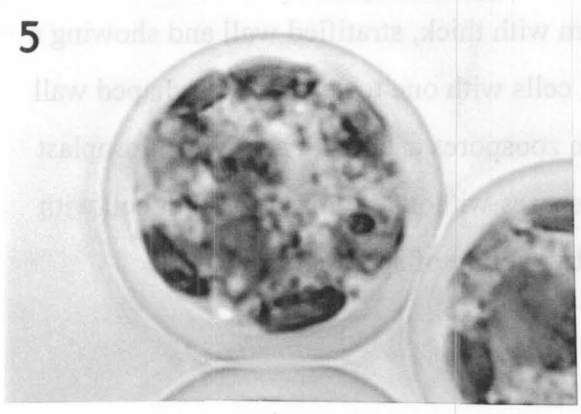
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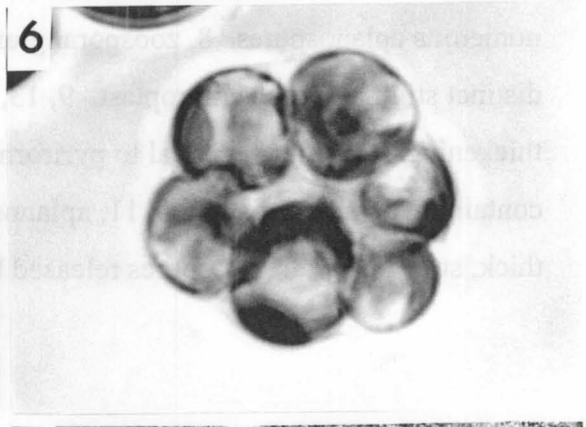

6


7
I
1μm

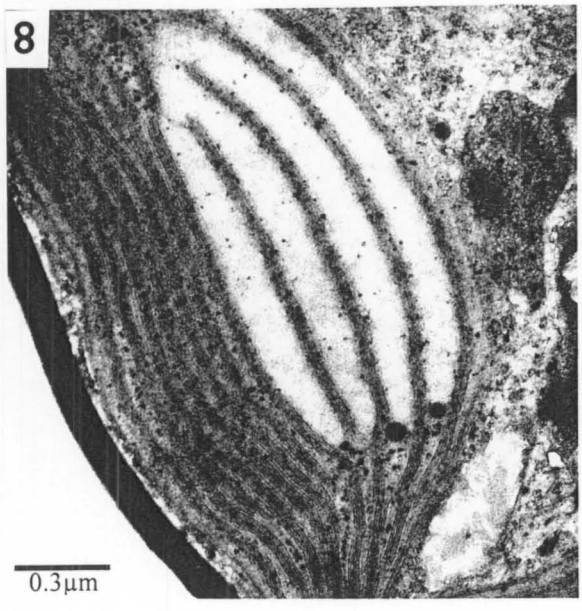

8
0.3μm

Fig. 23. *Botrydiopsis* morphotype B9.

1-2, young cells. 3-5, spherical cells showing spindle-shaped chloroplasts each containing a pyrenoid which bulges from the inner face of the chloroplast. 6, adult cell with vacuoles (V). 7, aplanosporangium with thick, stratified wall and containing numerous aplanospores. 8, zoosporangium with thick, stratified wall and showing a distinct stigma in each chloroplast. 9, 13, cells with one to several lens-shaped wall thickenings. 10, sub-spherical to pyriform zoospores each with a single chloroplast containing an anterior stigma. 11, aplanospores without stigma. 12, adult cell with thick, stratified wall. 14, spores released by rupture of sporangium wall.

Scale bar is 10 μm.

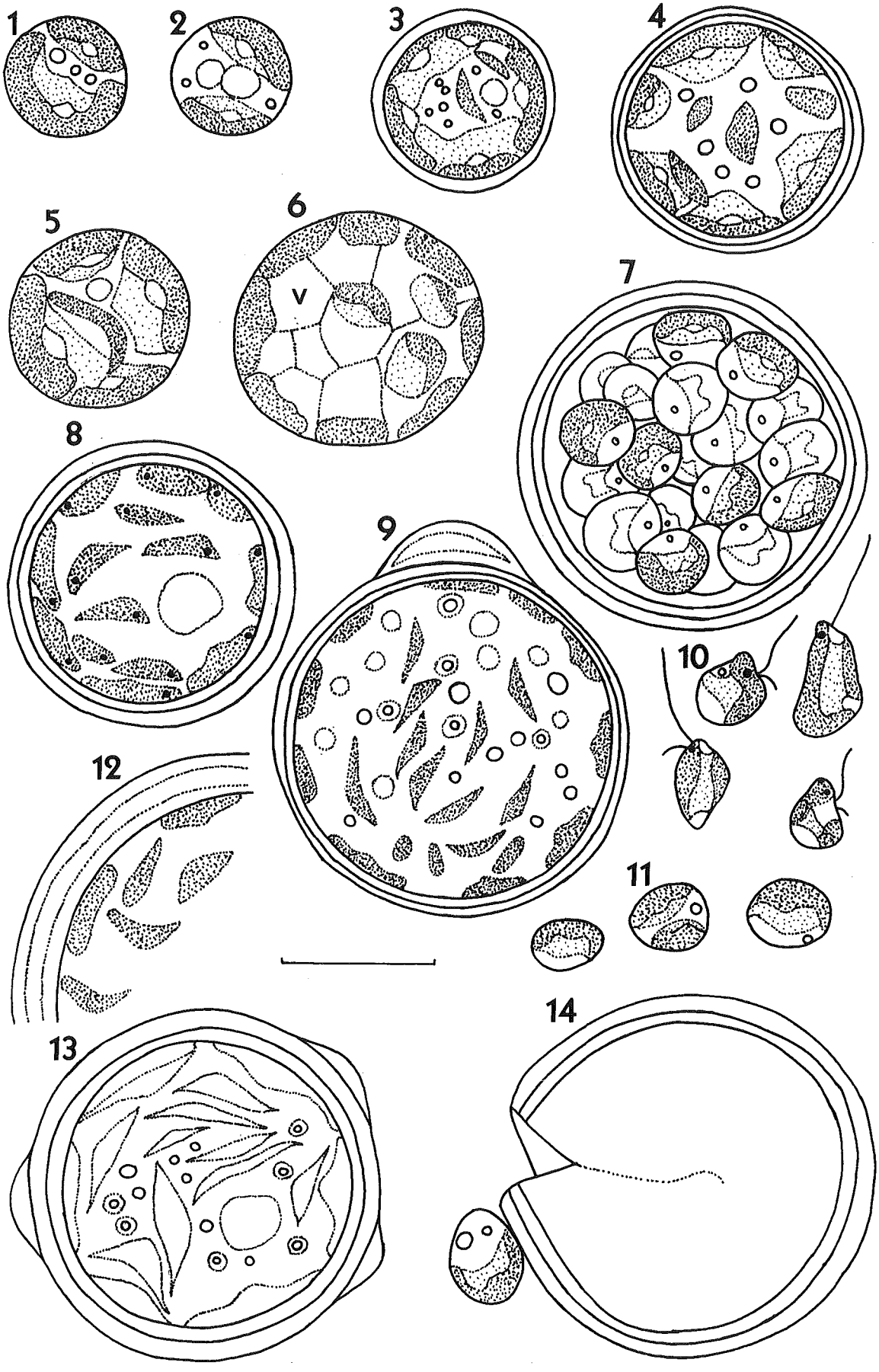
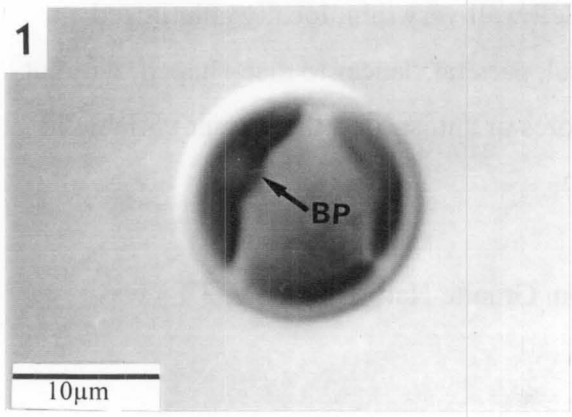
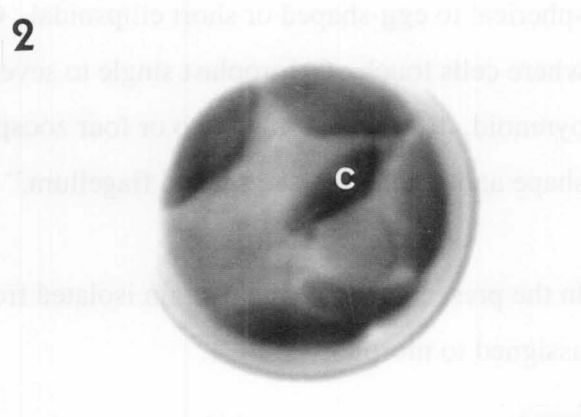

1
2
3
4
5
6
V
7
8
9
10
11
12
13
14

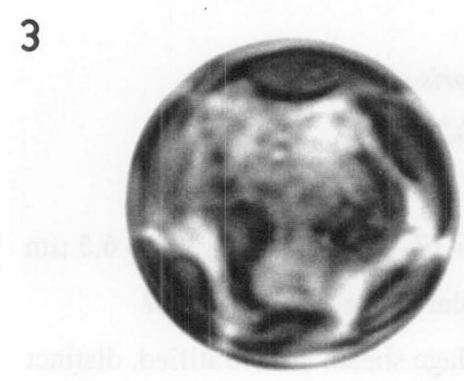
Fig. 24. *Botrydiopsis* morphotype B9, LM (1-6) and TEM (7, 8).

1, young cell with bulged pyrenoid (BP). 2, mature cell showing spindle-shaped chloroplast (C). 3, cell with bulged pyrenoid in each chloroplast. 4, cell with thick cell wall. 5, zoosporangium containing numerous zoospores and with thick wall. 6, aplanosporangium. 7, moderately fixed cell with bulged pyrenoid. 8, chloroplast showing thylakoid lamella passing through the centre of pyrenoid and lamellae interconnected at the end of pyrenoid (I).

Scale in 1 also applies to 2-6.


1
BP
10µm


2
C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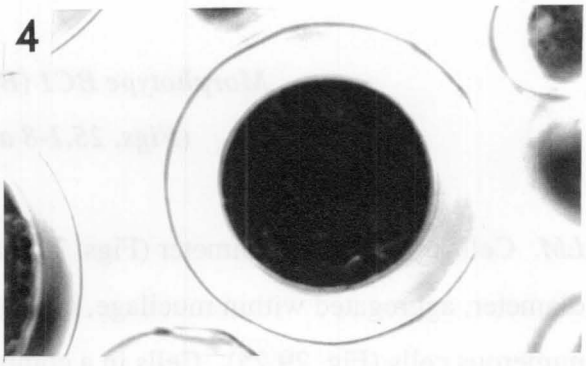
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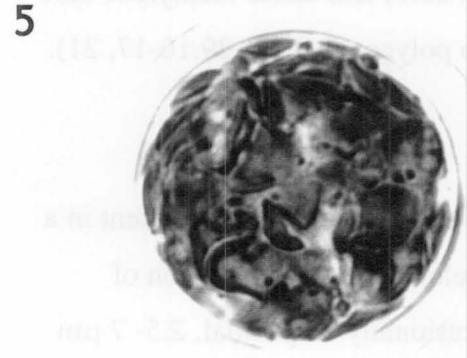
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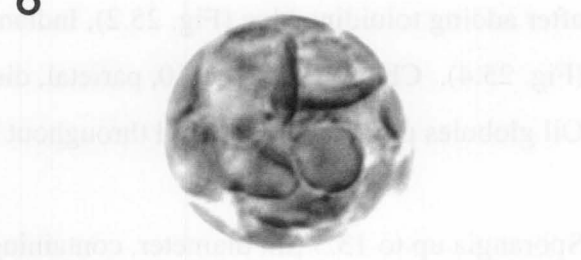
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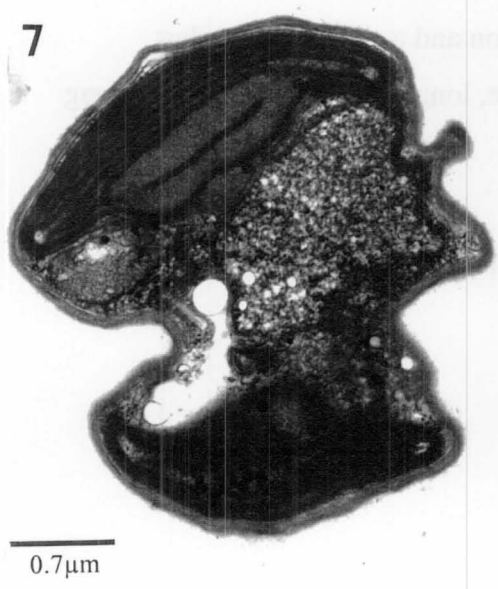
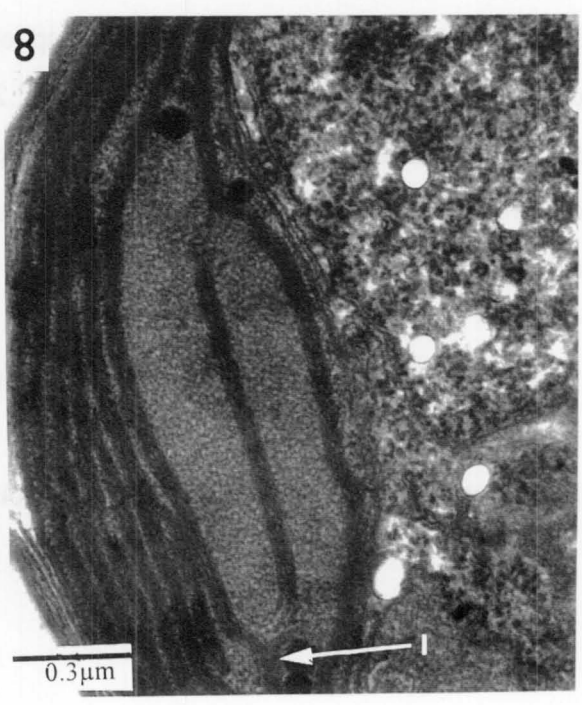

7
0.7µm


8
0.3µm
I

spherical to egg-shaped or short ellipsoidal. Cell wall very thin, locally gelatinized where cells touch. Chloroplast single to several, parietal, saucer to disc-shaped, without pyrenoid. Reproduction by two or four zoospores or autospores, the former variable in shape and with a reduced second flagellum."

In the present study a single strain isolated from Granite Harbour (Table. 3.1) was assigned to morphotype BC1.

Morphotype BC1 (Botryochloris sp. A)
(Figs. 25.1-8 and 29.16-25)

LM. Cells up to 15 μm diameter (Figs. 25.1 and 29.16-17, 21), but mostly up to 6.5 μm diameter, aggregated within mucilage, in dense, irregular clusters consisting of numerous cells (Fig. 29.25). Cells in a common mucilage sheath non-stratified, distinct after adding toluidine blue (Fig. 25.2), Indian ink (Fig. 25.3) and dilute methylene blue (Fig. 25.4). Chloroplasts 2 to 10, parietal, discoidal to polygonal (Figs. 29.16-17, 21). Oil globules numerous, scattered throughout the cell.

Sporangia up to 13.5 μm diameter, containing 2 to 22 spores, four spores frequent in a sporangium (Figs. 29.20, 22-24 and 25.5-7). Spores released by gelatinization of sporangium wall. Aplanospores mostly spherical, occasionally ellipsoidal, 2.5- 7 μm diameter, with one to two chloroplasts (Fig. 29.19). Zoospores 4-7 μm by 2.5-4.5 μm, fusiform to amoeboid, with a hyaline posterior region and a single chloroplast containing an anterior stigma, unequally biflagellate, long flagellum up to 8 μm long (Fig. 29.18).

TEM. Cells poorly fixed. Chloroplast lacking a pyrenoid (Fig. 25.8).

Distribution. Recorded from Granite Harbour.

Chlorellidium Vischer and Pascher 1937

Vischer (1937) established *Chlorellidium* with the following diagnosis (translated from the German):

"Colony usually consists of a tetrad, sometimes groups of two cells. Cells make contact, strongly flattened, joined together without space or with a small central space. Attached cells cannot be separated by pressure, even when they liberate zoospores or autospores they do not separate from each other. Occasionally scattered cells grow significantly but do not form tetrads, but form many zoospores or autospores. Cell wall thin or thick. Chloroplasts two to several or many, parietal, disc-shaped. Reproduction by zoospores or autospores. Sporangium ruptures by forming a large hole. Sporangium wall remains intact without gelatinization. Predominantly autospores are formed which after liberation remain combined in tetrads. Occasionally many (16 to 32) small autospores formed which do not stick to each other and remain isolated They grow to normal cell size and then again form groups of cells."

There are two species of *Chlorellidium* both of which are described from soil (Ettl, 1978; Ettl and Gärtner, 1995). In the present study eight strains were assigned to two morphotypes, C1 and C2 (Table. 3.1).

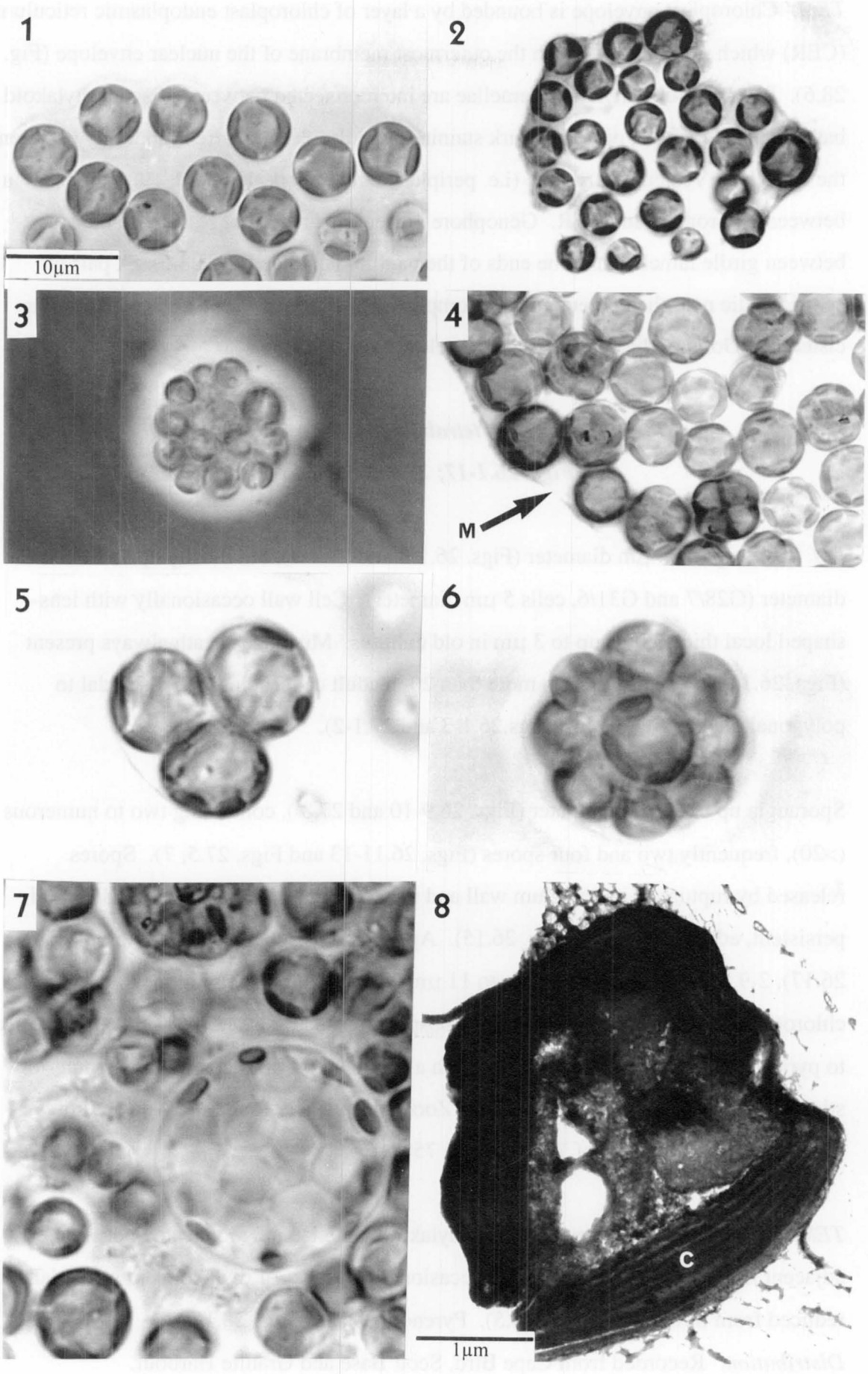
Features common to all morphotypes

LM. Colonies consist of solitary tetrads of cells (Figs. 26.5-7; 29.5, 11), sometimes aggregated into 2, 3, 4 tetrads (Figs. 26.4; 27.3-4; 29.4, 13 and 30.3-5). Cells spherical to ellipsoidal, multinucleate. Cell wall smooth, thin in young cultures but up to 2.5 µm thick, stratified in old cultures. Chloroplasts numerous, parietal, discoidal to polygonal shaped. Oil globules numerous, scattered throughout the cell. Vacuoles large, scattered throughout cell (Figs. 26.8; 27.6 and 29.12). Reproduction by aplanospores or zoospores or vegetative division. Zoospores naked, sub-spherical to pyriform to amoeboid, with two unequal flagella. Vegetative division forming tetrads of daughter cells, frequent.

Fig. 25. *Botryochloris* morphotype BC1, LM (1-7) and TEM (8).

1, group of cells surrounded by thin, indistinct mucilage. 2, group of cells enveloped by mucilage which has been studied with toluidine blue. 3, mucilage distinct after adding Indian ink. 4, part of a cluster of cells surrounded by mucilage (M) after adding dilute methylene blue which has stained the outer layer. 5, sporangium containing four spores in a tetrahedral arrangement. 6, aplanosporangium containing aplanospores. 7, sporangium with numerous vacuoles. 8, a poorly fixed (at room temperature) cell in which one chloroplast (C) can be seen to lack a pyrenoid.

Scale in 1 also applies to 2-7.


1
10µm
2
3
4
M
5
6
7
8
C
1µm

TEM. Chloroplast envelope is bounded by a layer of chloroplast endoplasmic reticulum (CER) which is continuous with the outermost membrane of the nuclear envelope (Fig. 28.6). Parallel, three-thylakoid lamellae are interconnected between adjacent thylakoid bands. Girdle lamella present. Dark staining lipid droplets occurred randomly between the lamellae. Vesicular structure (i.e. periplastidial network, Hibberd, 1980) appears in between chloroplast and CER. Genophore present in a region of less dense matrix between girdle lamellae and the ends of the parallel lamellae (Fig. 28.6). A pair of centrioles lie near the nucleus-plastid complex. Mitochondria scattered, with tubular cisternae. Golgi bodies lie against the nuclear envelope.

Morphotype C1 (C. tetrabotrys Vischer and Pascher)
(Figs. 26.1-17; 27.1-8 and 28.1-6)

LM. Cells up to 33 μm diameter (Figs. 26.1-3 and 27.1-2), but mostly up to 11 μm diameter (G28/7 and G31/6, cells 5 μm diameter). Cell wall occasionally with lens-shaped local thickenings up to 3 μm in old cultures. Mucilage sheath always present (Figs. 26.14, 18). Chloroplasts more than 20 in adult cells, spindle to discoidal to polygonal, lacking pyrenoids (Figs.26.1-3 and 27.1-2).

Sporangia up to 33 μm diameter (Figs. 26.9-10 and 27.8), containing two to numerous (>20), frequently two and four spores (Figs. 26.11-13 and Figs. 27.5, 7). Spores released by rupture of sporangium wall and not persistent (758, 898 and G28/7, wall persistent, adherent to spore, Fig. 26.15). Aplanospores spherical to ellipsoidal (Fig. 26.17), 2-9.5 μm diameter (757, up to 11 μm diameter), mostly with one to two chloroplasts, occasionally up to eight. Zoospores 1.5-7 μm by 1.5-4.5 μm, sub-spherical to pyriform to amoeboid (Fig. 26.16), with a single chloroplast containing an anterior stigma; long flagellum up to 7μm long. Zoospores usually found in 14 day cultures (found in 28 day cultures of 757, 597 and 758).

TEM. Chloroplast with parallel, three-thylakoid lamellae interconnected between adjacent thylakoid bands (Fig. 28.4), occasionally number of thylakoids in girdle lamella reduced from three to one (Fig. 28.5). Pyrenoid absent (Figs. 28.1-3).
Distribution: Recorded from Cape Bird, Scott Base and Granite Harbour.

Morphotype C2 (Chlorellidium sp. A)
(Figs. 29.1-15; 30.1-8 and 31.1-6)

LM. Cells up to 21.5 μm diameter (Figs. 29.1-3 and 30.1-2), but mostly about 12.5 μm diameter. Cell wall occasionally with lens-shaped local thickenings up to 1.5 μm wide in old cultures. Mucilage sheath thin, present in old cultures. Chloroplasts more than 20 in adult cells, discoidal to polygonal, each with a pyrenoid projecting as a bulge from the inner face of the chloroplast (Figs. 29.1-3 and 30.1-2).

Sporangia up to 33 μm diameter (Figs. 29.6-9 and 30.6-8), containing two to numerous (>20) spores, two and four spores frequent. Spores released by rupture of sporangium wall (Fig. 29.10). Aplanospores spherical to ellipsoidal (Fig. 29.15), 2.5- 12 μm diameter, mostly with one to two chloroplasts, occasionally up to six. Zoospores 3.5-7 μm by 2.5-5 μm, sub- spherical to pyriform to amoeboid (Fig. 29.14), with single chloroplast containing an anterior stigma; long flagellum up to 7μm long. Zoospores usually found in 14 day cultures (found in 28 day cultures of 871).

TEM. Chloroplast with three-thylakoid lamellae frequently interconnected between adjacent thylakoid bands in the pyrenoid (Figs. 31.1, 5) and also between thylakoids outside the pyrenoid (Figs. 31.2, 6). Pyrenoid projecting as a bulge from the inner face of the chloroplast, traversed by thylakoid lamellae (Figs. 31.3-4). No reduction in the number of thylakoids in girdle lamella like C1.

There are two types of pyrenoid in this morphotype: 1) strain 785, thylakoid lamellae are frequently interconnected in the pyrenoid, 2) strain 871, thylakoid lamellae are interconnected outside the pyrenoid.

Distribution: Recorded from Scott base and Christchurch.

Fig. 26. *Chlorellidium* morphotype C1.

1, young cell. 2-3, spherical to ellipsoidal adult cells showing discoidal to polygonal chloroplasts which lack a pyrenoid. 4, colony composed of numerous adherent tetrads. 5-7, tetrads with different cell arrangements. 5, tetrahedral tetrad. 6-7, isobilateral tetrads. 8, adult cell with a large vacuole. 9, zoosporangium showing a distinct stigma in each chloroplast. 10, aplanosporangium contains numerous aplanospores. 11, 13, aplanosporangium with two aplanospores. 12, aplanosporangium with four aplanospores. 14, 18, tetrad with thin mucilage sheath. 15, strains 758, 898 and G28/7, sporangium wall persistent and adherent to spores. 16, sub-spherical, pyriform to amoeboid zoospores each with a single chloroplast containing an anterior stigma. 17, aplanospores with and without stigma.

Scale bar is 10 μm.

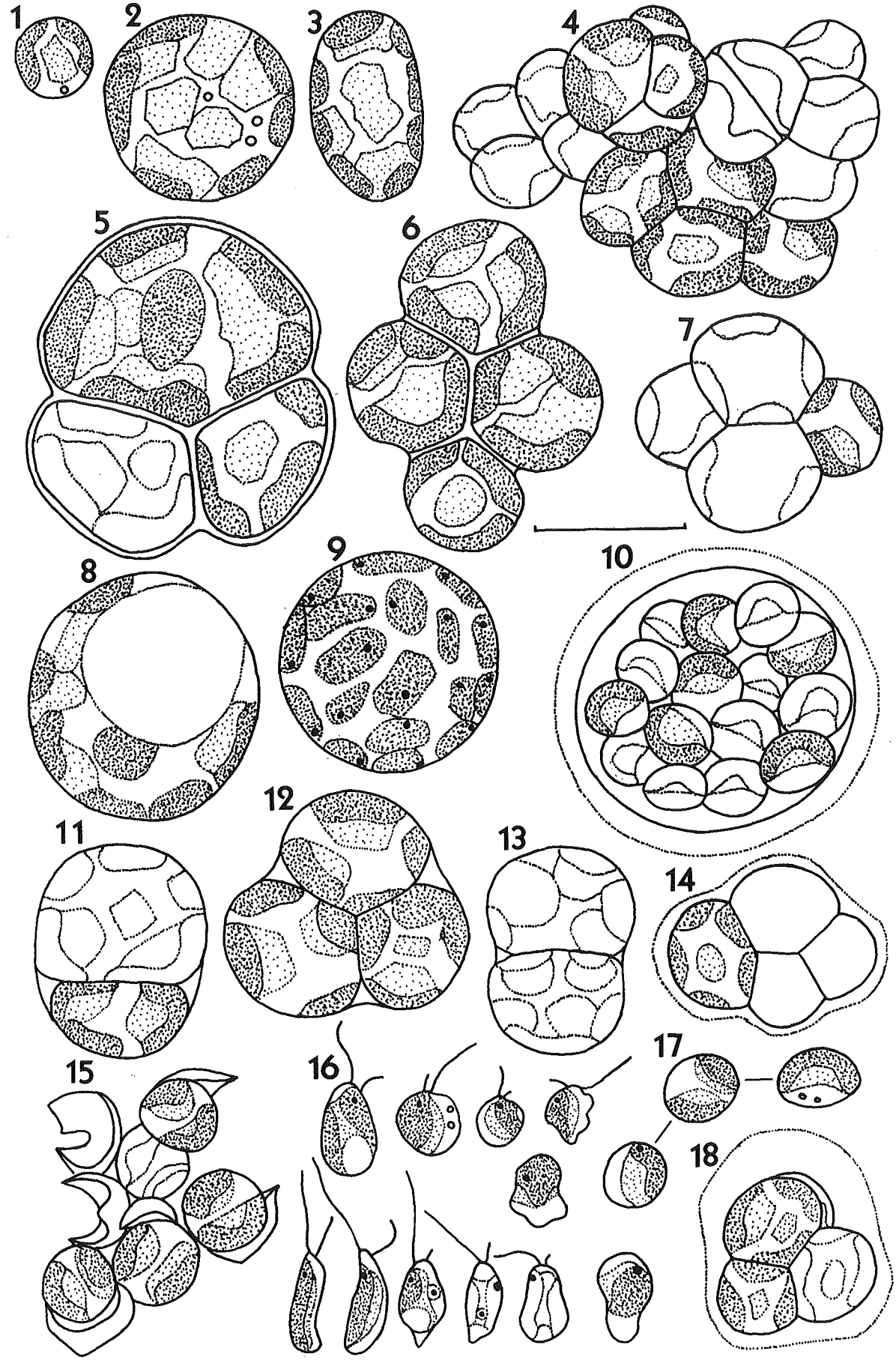

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Fig. 27. *Chlorellidium* morphotype C1, LM.

1, young cell. 2, adult cell without pyrenoid. 3, tetrahedral tetrad. 4, single tetrad (TE) and adherent vegetative cells. 5, aplanosporangium with four aplanospores. 6, young cells, one cell with large vacuole (V). 7, aplanosporangium with two aplanospores. 8, zoosporangium showing distinct stigma (ST) in each chloroplast.

Scale in 1 also applies to 2-8.

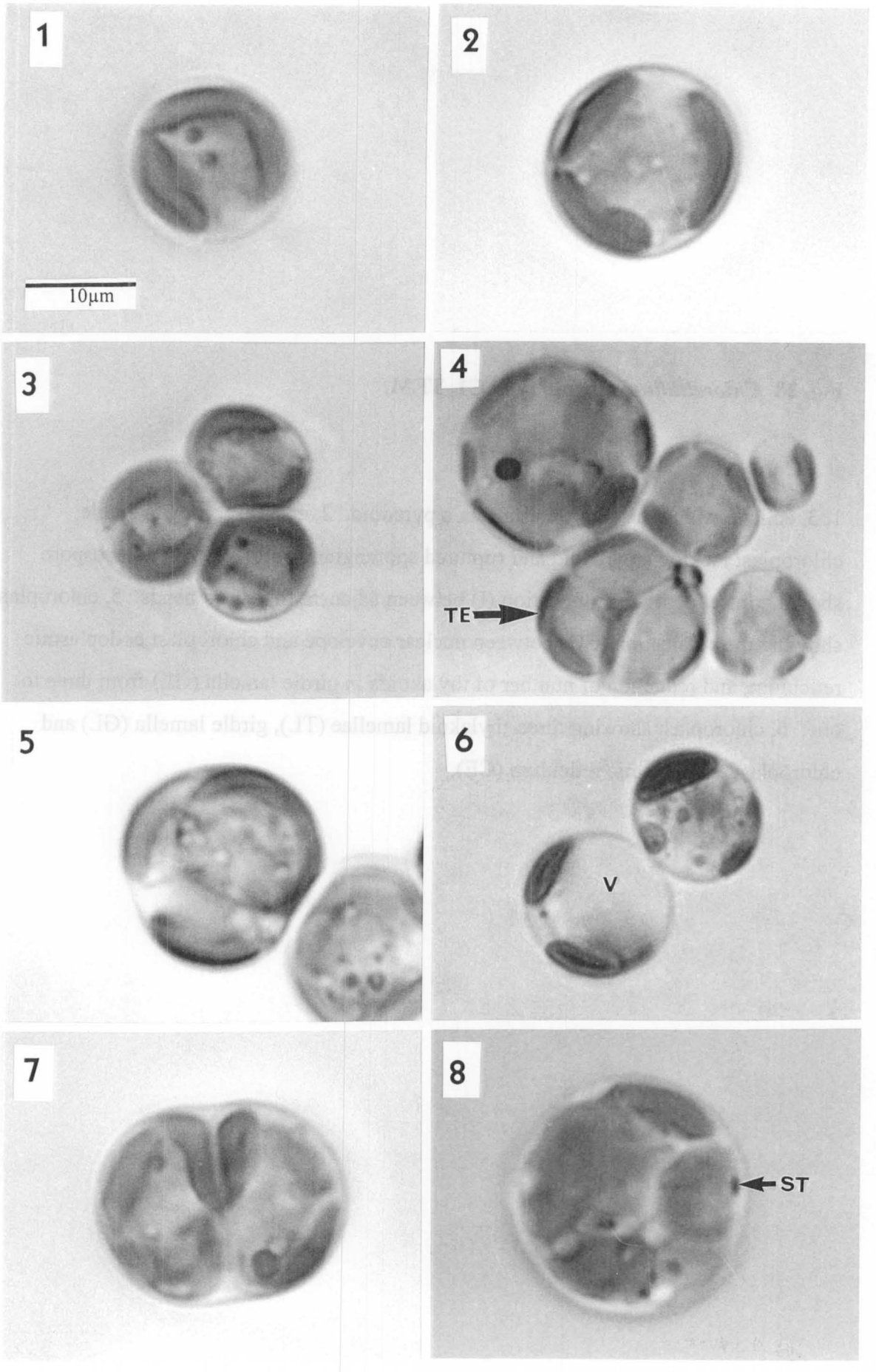
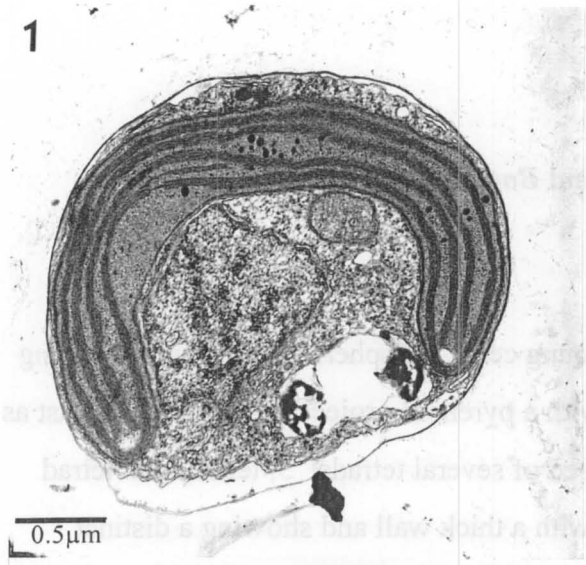
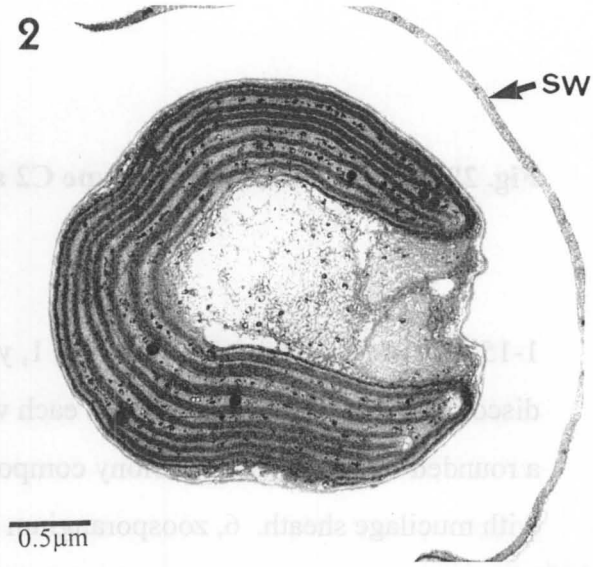
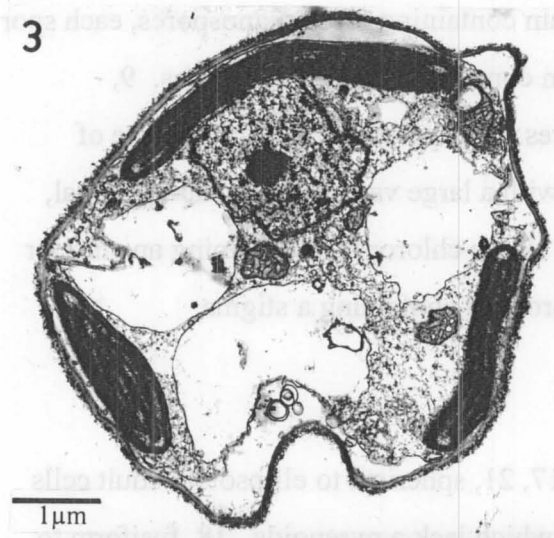

1
10µm
2
3
4
TE
5
6
V
7
8
ST

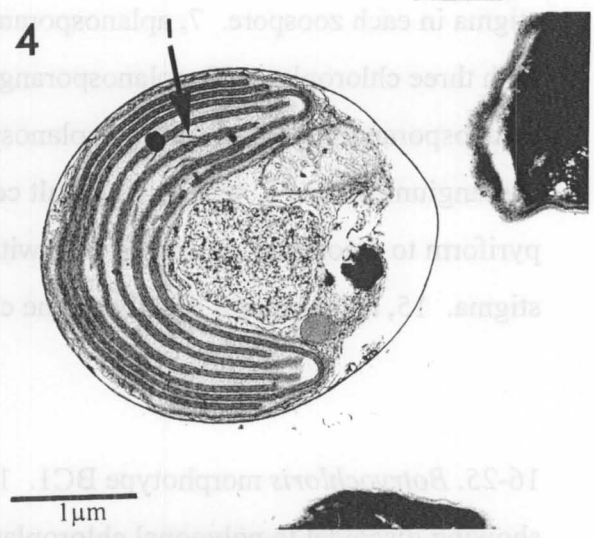
Fig. 28. *Chlorellidium* morphotype C1, TEM.

1, 3, cells in which the chloroplasts lack a pyrenoid. 2, aplanospore with single chloroplast lacking a pyrenoid and ruptured sporangium wall (SW). 4, aplanospore showing infrequent interconnection (I) between adjacent thylakoid bands. 5, chloroplast showing the connection (CO) between nuclear envelope and chloroplast endoplasmic reticulum, and reduction of number of thylakoids in girdle lamella (GL) from three to one. 6, chloroplast showing three-thylakoid lamellae (TL), girdle lamella (GL) and chloroplast endoplasmic reticulum (CE).


1
0.5µm


2
SW
0.5µm


3
1µm


4
1µm


5
CO
GL
0.3µm

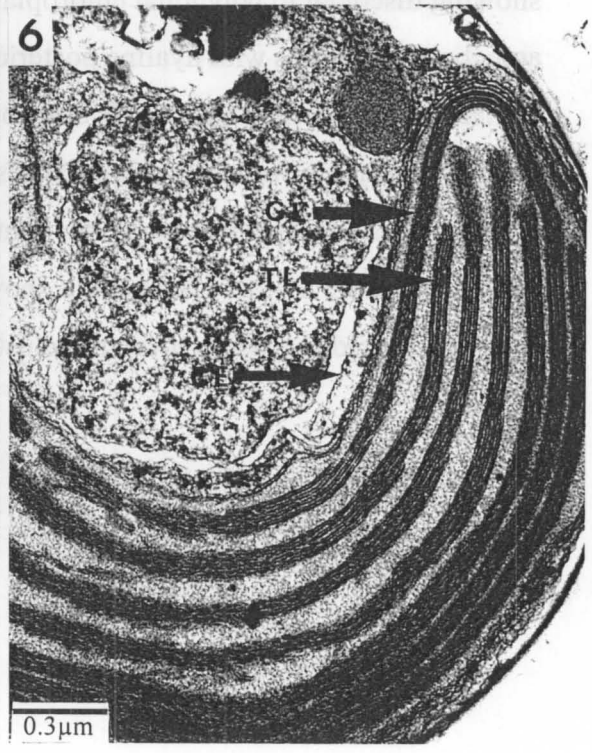
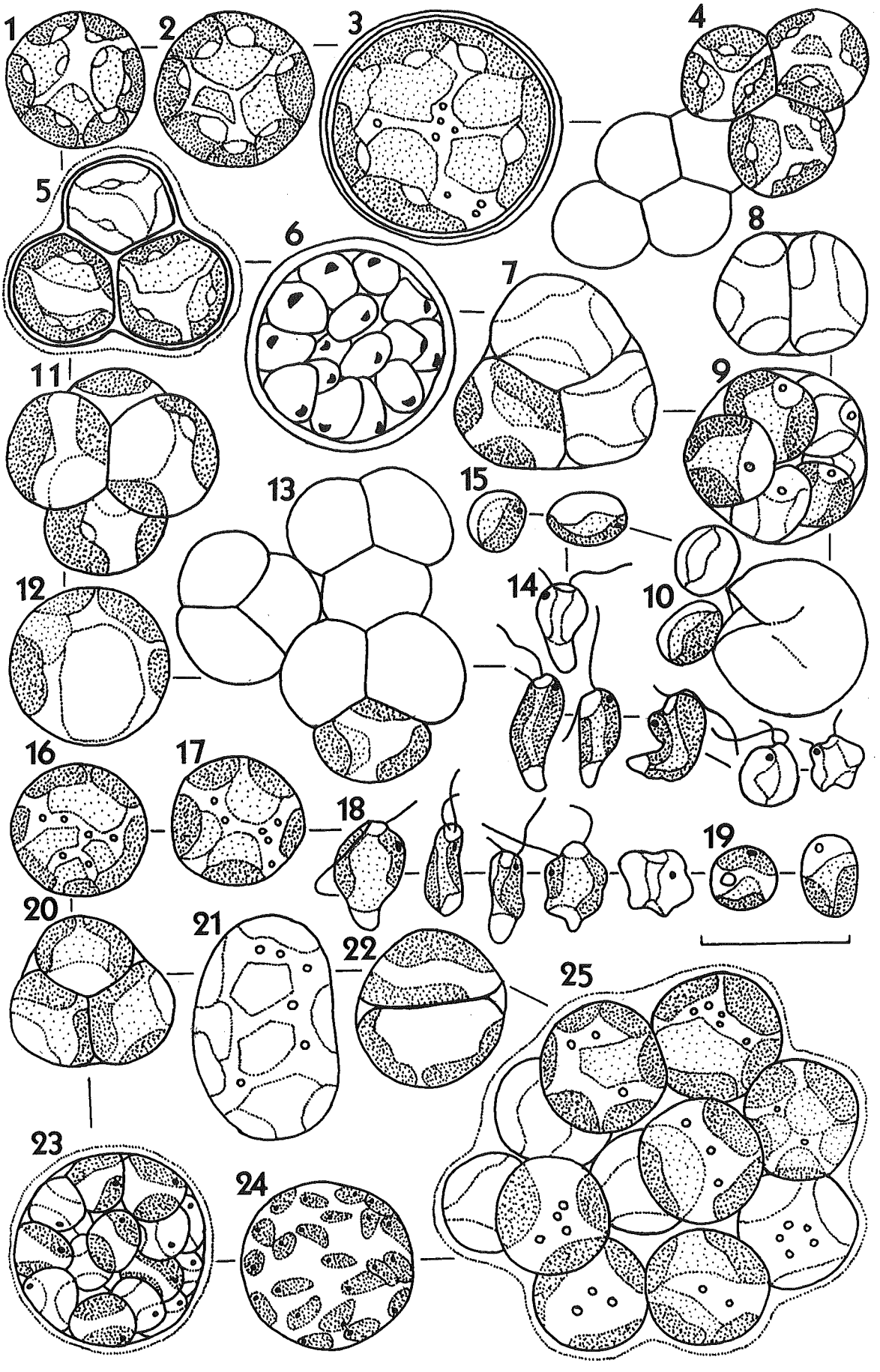

6
GL
TL
CL
0.3µm

Fig. 29. *Chlorellidium* morphotype C2 and *Botryochloris* morphotype BC1.

1-15. *Chlorellidium* morphotype C2. 1, young cell; 2-3, spherical adult cells showing discoidal to polygonal chloroplasts each with a pyrenoid projecting from chloroplast as a rounded bulge. 4, 12-13, colony composed of several tetrads. 5, tetrahedral tetrad with mucilage sheath. 6, zoosporangium with a thick wall and showing a distinct stigma in each zoospore. 7, aplanosporangium containing four aplanospores, each spore with three chloroplasts. 8, aplanosporangium containing two aplanospores. 9, aplanosporangium containing six aplanospores. 10, spores released by rupture of sporangium wall. 11, tetrad. 12, adult cell with a large vacuole. 14, sub-spherical, pyriform to amoeboid zoospores each with a single chloroplast containing an anterior stigma. 15, aplanospores each with one chloroplast containing a stigma.

16-25. *Botryochloris* morphotype BC1. 16-17, 21, spherical to ellipsoidal adult cells showing discoidal to polygonal chloroplasts which lack a pyrenoids. 18, fusiform to amoeboid zoospores with hyaline posterior end and each with a single chloroplast containing an anterior stigma. 19, aplanospores with and without stigma. 20, aplanosporangium with four aplanospores. 22, aplanosporangium containing two aplanospores. 23, aplanosporangium containing numerous spores and with a thin mucilage sheath. 24, zoosporangium showing a distinct stigma in each chloroplast. 25, cells aggregated in a dense, irregular cluster and held within mucilage.

Scale bar is 10 μm.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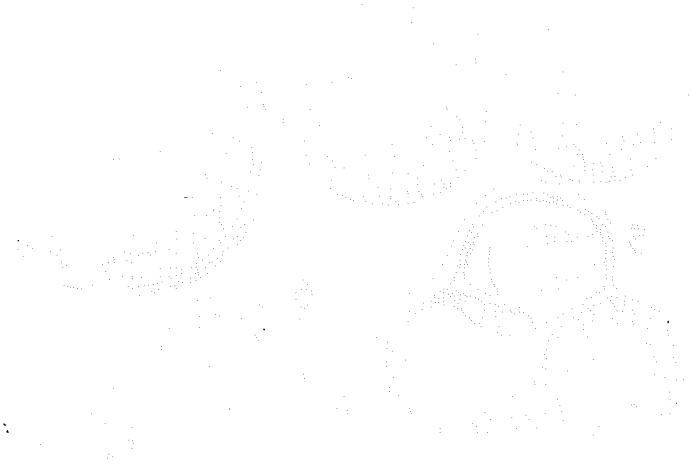


Fig. 30. *Chlorellidium* morphotype C2, LM.

1, adult cell showing bulged pyrenoid (BP). 2, group of young cells. 3, two tetrads. 4, tetrahedral tetrads. 5, group of tetrads. 6, aplanosporangium with two aplanospores. 7, aplanosporangium containing eight aplanospores. 8, aplanosporangium containing four aplanospores.

Scale in 1 also applies to 2-8.

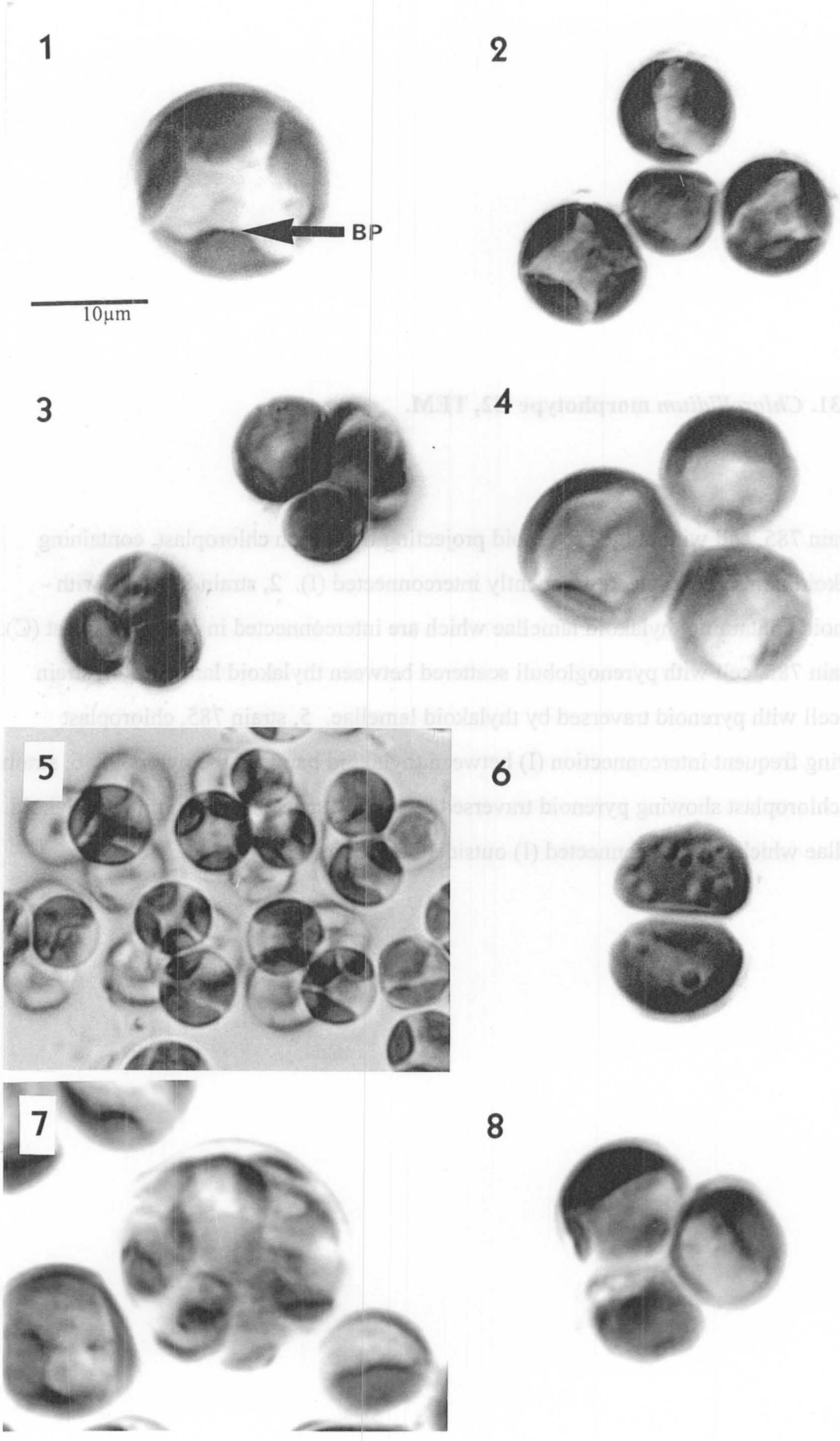
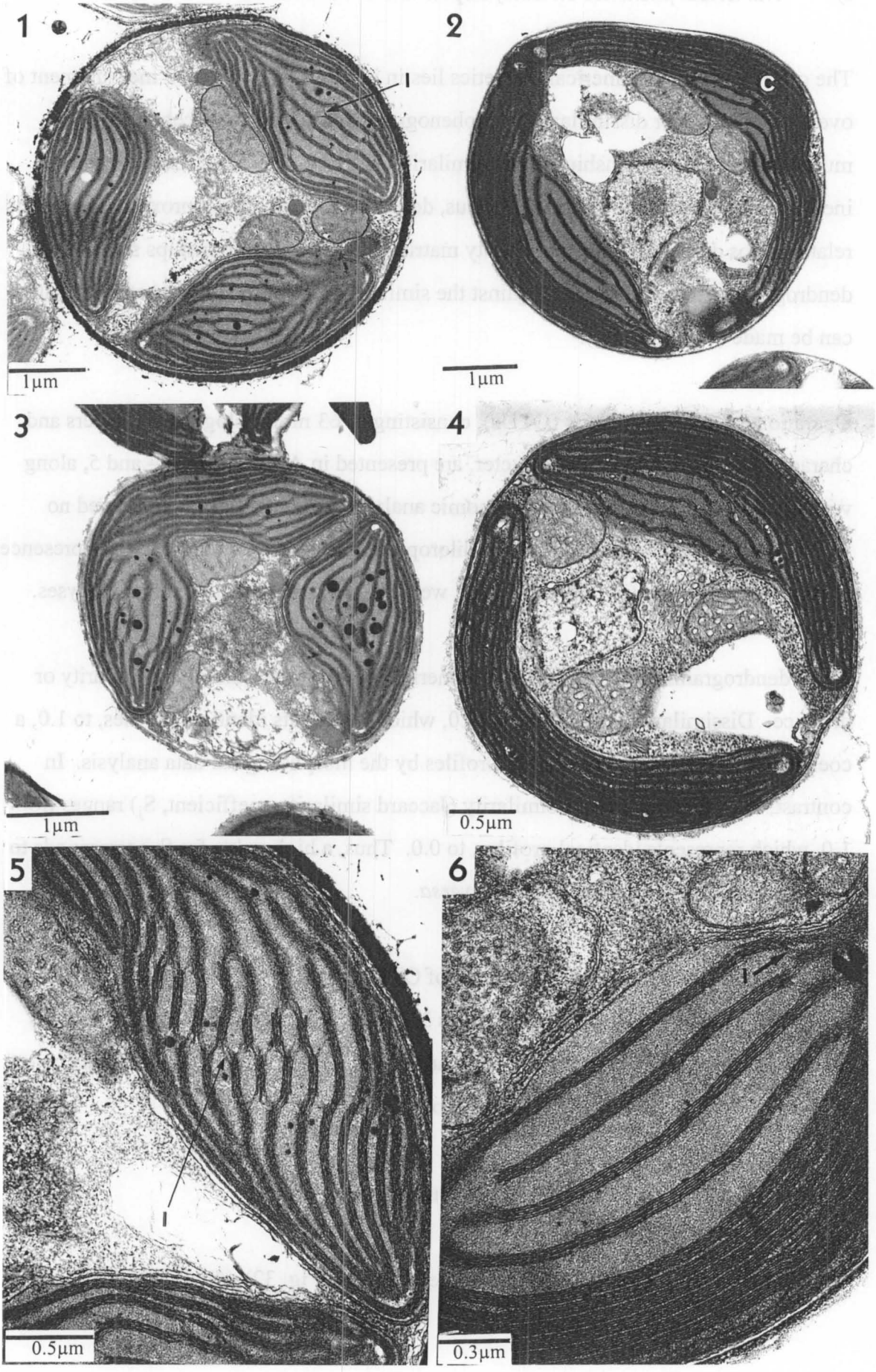

1
BP
10µm
2
3
4
5
6
7
8

Fig. 31. *Chlorellidium* morphotype C2, TEM.

1, strain 785, cell with bulged pyrenoid projecting from each chloroplast, containing thylakoid lamellae which are frequently interconnected (I). 2, strain 871, cell with pyrenoid containing thylakoid lamellae which are interconnected in one chloroplast (C). 3, strain 785, cell with pyrenoglobuli scattered between thylakoid lamellae. 4, strain 871, cell with pyrenoid traversed by thylakoid lamellae. 5, strain 785, chloroplast showing frequent interconnection (I) between thylakoid bands in the pyrenoid. 6, strain 871, chloroplast showing pyrenoid traversed by widely spaced parallel, three-thylakoid lamellae which are interconnected (I) outside the pyrenoid.


1
I
1µm
2
C
1µm
3
1µm
4
0.5µm
5
I
0.5µm
6
I
0.3µm

c) Numerical phenetics on Botrydiopsis and Chlorellidium

The greatest value of numerical phenetics lies in its relatively objective measurement of overall similarity (or dissimilarity). A phenogram/dendrogram reduces the multidimensional relationships of the similarity matrix to two dimensions, which inevitably involves some distortion. Thus, dendrograms are only approximations of the relationships derivable from a similarity matrix. Therefore, relationships shown in a dendrogram need to be checked against the similarity matrix before phenetic groupings can be made (Ward, 1993b).

Operational Taxonomic Units (OTUs), consisting of 63 morphological characters and character state codes for each character, are presented in Appendices 3, 4 and 5, along with the data matrix used for the taxonomic analyses. Characters which showed no variation between strains, e.g. parietal chloroplast, presence of oil globules and presence of vegetative division (in *Chlorellidium*), were omitted from the taxonomic analyses.

In the dendrograms (Figs. 32, 33), the numerical axis is a measure of dissimilarity or distance. Dissimilarity is scored from 0.0, which represents identical profiles, to 1.0, a coefficient illustrating no sharing of profiles by the morphological data analysis. In contrast, the value of pairwise similarity (Jaccard similarity coefficient, S_j) ranges from 1.0, which represents identical profiles, to 0.0. Thus, a high value for S_j corresponds to a small value for dissimilarity and *vice versa*.

The overall similarity between every pair of OTUs using Jaccard similarity coefficient (S_j) is shown in a similarity matrix (Tables 3.3, 3.4). A coefficient of 0.80 or more is taken as representing a high level of similarity, 0.75 to 0.79 a moderate level and below 0.75 a low level of similarity (Ward, 1993b).

Botrydiopsis

The cluster analysis produced a stepwise dendrogram (Fig. 32) which indicates a high

level of similarity between the 27 strains of *Botrydiopsis*. Five clusters could be distinguished above 0.35 dissimilarity level. Main distinguishing characteristics of all these clusters and subclusters are given in Fig. 32.

Cluster A contains the single morphotype B5, represented by the single strain 836 from Victoria Valley. The similarity value (S_j) between strain 836 and each of the other strains ranges from 0.53 to 0.75 (Table. 3.3).

Two morphotypes B9 and B7 form a distinct cluster B. This cluster links with clusters C-E at lower levels of similarity, i.e. S_j values between each strain from cluster B and each strain from each clusters C-E range from 0.56 to 0.73.

Cluster C contains morphotype B1 (six strains) and B2 (six strains). It is associated with clusters D and E. The S_j values from pairwise comparisons between each strain from cluster C and each strain from clusters D and E ranges from 0.59 to 0.76, i.e. they have low to moderate similarity.

Cluster D contains morphotypes B6 (seven strains) and B8 (eight strains) which link with cluster E. Morphotypes B3 and B4, each represented by a single strain, form cluster E. The similarity values between each strain of cluster D and each strain of cluster E ranges from 0.63 to 0.84.

In addition, divisions within clusters B-E are at higher levels of similarity. Cluster B is divided into two subclusters. Morphotypes B9 and B7 form these subclusters although they have a higher S_j value of 0.86. Morphotypes B1 and B2 fall into two separate groups in cluster C. Comparisons of strains within C have S_j value 0.87. Cluster D is further divided into two subclusters although all strains in subclusters have higher S_j value 0.84. Similarly, cluster E is divided into two subclusters. These subclusters are represented by morphotypes B3 and B4. All strains in cluster E have S_j value 0.84.

Table 3.3. Pairwise comparisons of similarity coefficients based on morphological characteristics of 27 strains of *Botrydiopsis*.

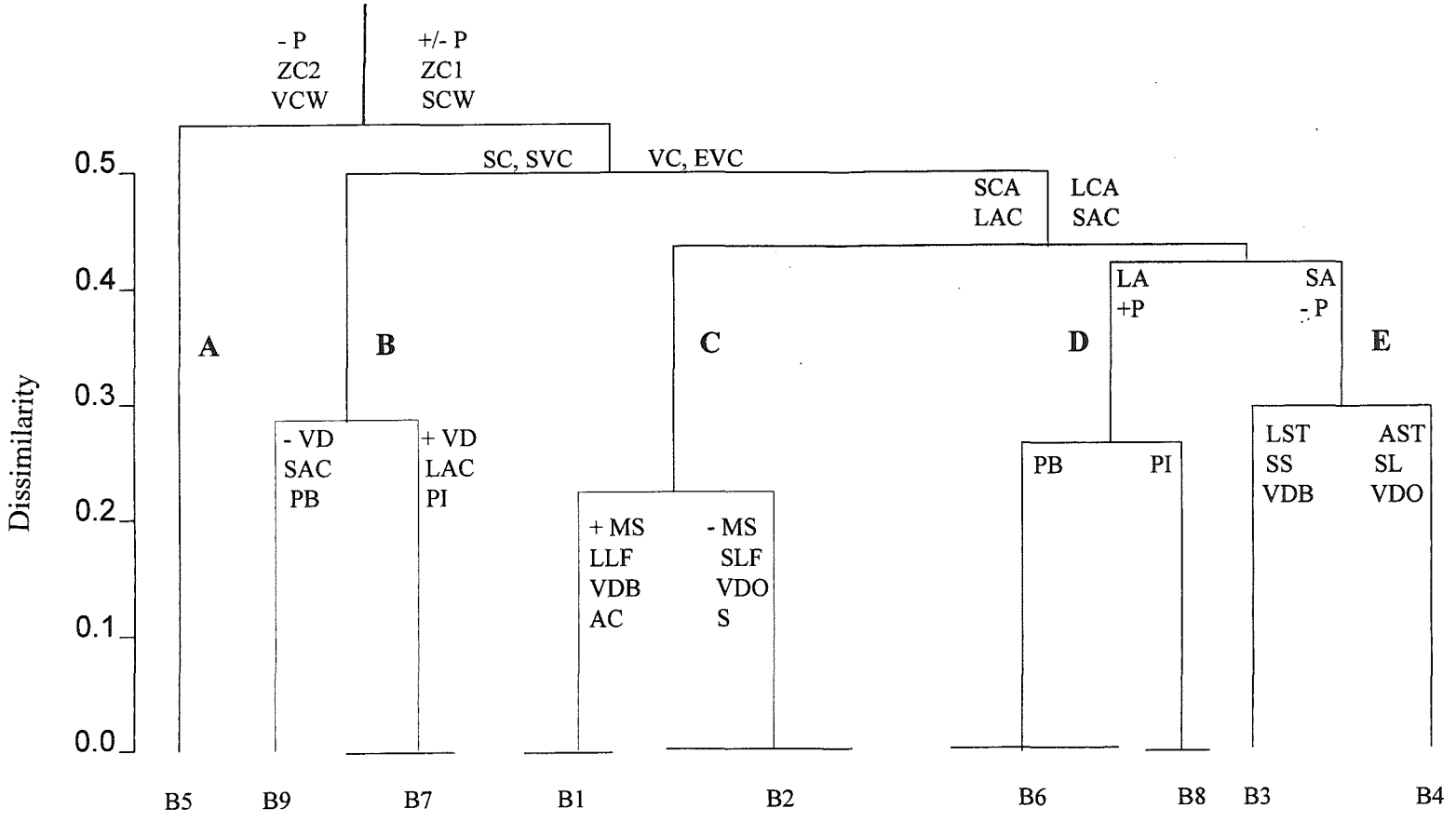
Morphotype	Strain	Morphotype	B1					B2						B3	B4	B5	B6							B7		B8		B9
		Strain	645	894	829	801	G41/6	WI/1	895	896	897	886	G12/1	724	485	G94/1	836	837	G9/8	G19/2	G24/8	G27/1	G31/5	G99/2	877	638	864	G27/2	908
B1	645		-	1	1	1	1	1	0.87	0.87	0.87	0.87	0.87	0.87	0.69	0.63	0.60	0.68	0.68	0.68	0.68	0.68	0.68	0.67	0.67	0.59	0.59	0.65
	894			-	1	1	1	1	0.87	0.87	0.87	0.87	0.87	0.87	0.69	0.63	0.60	0.68	0.68	0.68	0.68	0.68	0.68	0.67	0.67	0.59	0.59	0.65
	829				-	1	1	1	0.87	0.87	0.87	0.87	0.87	0.87	0.69	0.63	0.60	0.68	0.68	0.68	0.68	0.68	0.68	0.67	0.67	0.59	0.59	0.65
	801					-	1	1	0.87	0.87	0.87	0.87	0.87	0.87	0.69	0.63	0.60	0.68	0.68	0.68	0.68	0.68	0.68	0.67	0.67	0.59	0.59	0.65
	G41/6						-	1	0.87	0.87	0.87	0.87	0.87	0.87	0.69	0.63	0.60	0.68	0.68	0.68	0.68	0.68	0.68	0.67	0.67	0.59	0.59	0.65
	WI/1							-	0.87	0.87	0.87	0.87	0.87	0.87	0.69	0.63	0.60	0.68	0.68	0.68	0.68	0.68	0.68	0.67	0.67	0.59	0.59	0.65
B2	895								-	1	1	1	1	1	0.76	0.73	0.63	0.75	0.75	0.75	0.75	0.75	0.75	0.63	0.63	0.62	0.62	0.59
	896									-	1	1	1	1	0.76	0.73	0.63	0.75	0.75	0.75	0.75	0.75	0.75	0.63	0.63	0.62	0.62	0.59
	897										-	1	1	1	0.76	0.73	0.63	0.75	0.75	0.75	0.75	0.75	0.75	0.63	0.63	0.62	0.62	0.59
	886											-	1	1	0.76	0.73	0.63	0.75	0.75	0.75	0.75	0.75	0.75	0.63	0.63	0.62	0.62	0.59
	G12/1												-	1	0.76	0.73	0.63	0.75	0.75	0.75	0.75	0.75	0.75	0.63	0.63	0.62	0.62	0.59
	724													-	0.76	0.73	0.63	0.75	0.75	0.75	0.75	0.75	0.75	0.63	0.63	0.62	0.62	0.59
B3	485														-	0.84	0.75	0.76	0.76	0.76	0.76	0.76	0.76	0.56	0.56	0.67	0.67	0.60
B4	G94/1															-	0.75	0.67	0.67	0.67	0.67	0.67	0.67	0.56	0.56	0.63	0.63	0.60
B5	836																-	0.63	0.63	0.63	0.63	0.63	0.63	0.56	0.56	0.62	0.62	0.53
B6	837																	-	1	1	1	1	1	0.69	0.69	0.84	0.84	0.65
	G9/8																		-	1	1	1	1	0.69	0.69	0.84	0.84	0.65
	G19/2																			-	1	1	1	0.69	0.69	0.84	0.84	0.65
	G24/8																				-	1	1	0.69	0.69	0.84	0.84	0.65
	G27/1																					-	1	0.69	0.69	0.84	0.84	0.65
	G31/5																						-	0.69	0.69	0.84	0.84	0.65
	G99/2																							0.69	0.69	0.84	0.84	0.65
B7	877																							-	1	0.73	0.73	0.86
	638																								-	0.73	0.73	0.86
B8	864																									-	0.84	0.68
	G27/2																										-	0.59
B9	908																											-

Table 3.4. Pairwise comparisons of similarity coefficients based on morphological characteristics of eight strains of *Chlorellidium.*

Morphotype	Strain	Morphotype							
		C1						C2	
		Strain							
		597	757	758	898	G28/7	G31/6	785	871
C1	597	-	1	1	1	1	1	0.83	0.83
	757		-	1	1	1	1	0.83	0.83
	758			-	1	1	1	0.83	0.83
	898				-	1	1	0.83	0.83
	G28/7					-	1	0.83	0.83
	G31/6						-	0.83	0.83
C2	785							-	1
	871								-

Fig. 32. Cluster analysis dendrogram using similarity coefficient with average linkage based on morphological characteristics of 27 strains of *Botrydiopsis*. The scale on the Y-axis represents the degree of dissimilarity on a scale of 0 to 1. Bold letters in the figure indicate clusters referred to in the text. Abbreviations indicate main distinguishing features of clusters which are as follows:

+	presence of character
-	absence of character
AC	ability to cluster
S	single cell
LLF	greater length of longer flagellum
SLF	shorter length of longer flagellum
EVC	spherical to ellipsoidal vegetative cell
SVC	spherical vegetative cell
SC	spindle-shaped to discoidal chloroplast
VC	variably shaped chloroplast, e.g. spindle-shaped, discoidal, ellipsoidal to polygonal chloroplast
LA	larger aplanospore
SA	smaller aplanospore
LAC	larger adult cell
SAC	smaller adult cell
LCA	larger number of chloroplasts in aplanospore
SCA	smaller number of chloroplasts in aplanospore
LST	lens-shaped thickening
AST	absence of lens-shaped thickening
MS	mucilage sheath
P	pyrenoid
PB	bulged pyrenoid
PI	immersed pyrenoid
SL	larger sporangium
SS	smaller sporangium
VCW	verrucose cell wall
SCW	smooth cell wall
VD	vegetative division
VDO	vegetative division absent in old culture
VDB	vegetative division present in both young and old cultures
ZC1	mostly one, occasionally two chloroplasts in zoospore
ZC2	two chloroplasts in zoospore


- P
ZC2
VCW
+/- P
ZC1
SCW
SC, SVC
VC, EVC
SCA
LAC
LCA
SAC
LA
+P
SA
- P
Dissimilarity
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0.4
0.3
0.2
0.1
0.0
A
B
C
D
E
- VD
SAC
PB
+ VD
LAC
PI
+ MS
LLF
VDB
AC
- MS
SLF
VDO
S
PB
PI
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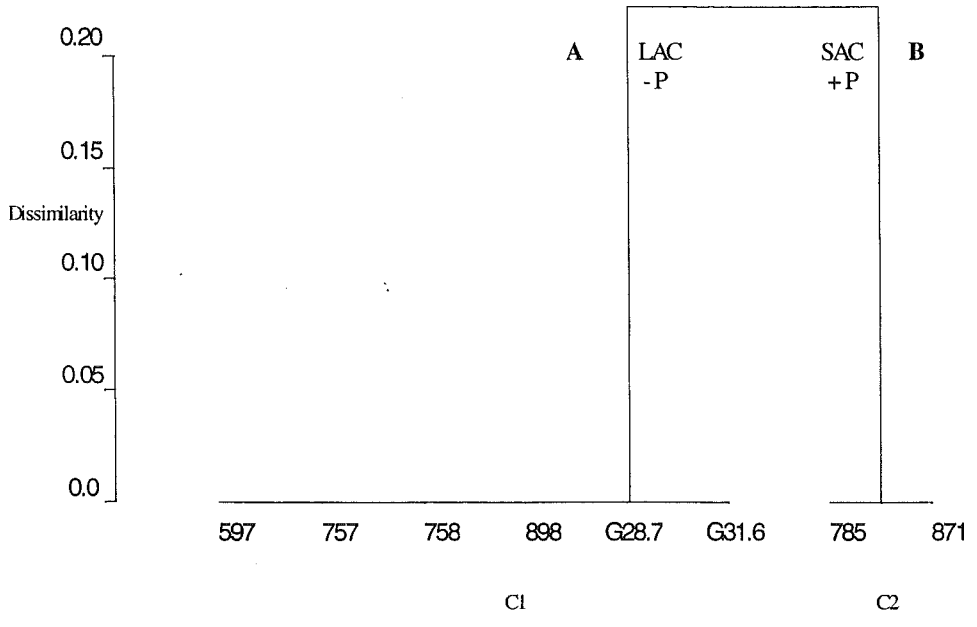


Fig. 33. Cluster analysis dendrogram using similarity coefficient with average linkage based on morphological characteristics of eight strains of *Chlorellidium*. The scale on the Y-axis represents the degree of dissimilarity on a scale of 0 to 1. Bold letters in the figure indicate clusters referred to in the text. Abbreviations indicate main distinguishing features of clusters which are as follows:

+	presence of character
-	absence of character
P	pyrenoid
LAC	larger adult cell
SAC	smaller adult cell

Chlorellidium

Two clusters, A and B could be distinguished at 0.2 dissimilarity level (Fig. 33). Main distinguishing features of these clusters are given in Fig. 33. Clusters A and B contain six and two strains, assigned in morphotypes C1 and C2 respectively. The similarity

values between each strain from cluster A and each strain from cluster B is 0.83 (Table. 3.4).

3.2.2 Isozyme analysis of Botrydiopsis, Botryochloris and Chlorellidium

a) Introduction

Since the enzyme electrophoretic technique was developed, it has been increasingly used to provide useful information in a wide range of biological situations. It is used for determining interspecific relationship, geographic variation within species, population sub-division as well as for analysis of systematic relationships (Hillis *et al.*, 1996; Manhart and McCourt, 1992). An advantage of electrophoretic data over morphological data is the ability to clarify and distinguish between ambiguous species where the only morphological features are nearly or completely indistinguishable, such as sibling species, cryptic species and species complex (Hillis, 1987). However, it is clear that molecular data are most useful when they are combined with data on other types of characters such as morphological ones (Buchheim and Chapman, 1992; Donoghue and Sanderson, 1992).

b) Establishing the technique

Initially, enzyme electrophoresis was tested to determine whether it gave similar profiles for genetically identical samples. This was done using multiple isolates from a single clonal culture of *Botrydiopsis* morphotype B7. All isolates gave identical banding patterns. This result confirmed that genetically identical isolates gave identical isozyme banding patterns and hence provided confidence in the technique.

c) Descriptions of isozyme banding patterns

Character matrices were formed representing presence or absence of bands at all putative loci of *Botrydiopsis, Chlorellidium* and *Botryochloris*, and are presented in Tables 3.5 and 3.6. Of the nine enzyme systems tested, four produced scorable banding

Table 3.5. [a]Presence or absence of stained protein bands obtained following starch gel electrophoresis of protein extracts from strains of *Botrydiopsis.*

[b]Enzyme	Band position	Morphotype B1						B2						B3	B4
		Strain 645	894	829	801	G41/6	WI/1	895	896	897	886	G12/1	724	485	G94/1
PGI	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	1	1	1	0	0	0	0	0	0	0	0	0	1	0
	4	0	0	0	1	0	0	1	0	0	1	0	0	1	0
	5	0	0	0	0	1	1	0	0	1	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G6PDH	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LAP	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SKDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 3.5. (cont.)

[b]Enzyme	Band positio n	Morphotype B5	B6							B7		B8		B9
		Strain 836	837	G9/8	G19/2	G24/8	G27/1	G31/5	G99/2	877	638	864	G27/2	908
PGI	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	1	0	0	0	0	0	0	0	0	0
	4	0	0	0	1	0	0	0	0	1	0	0	0	0
	5	1	0	1	0	0	0	0	0	1	1	0	1	0
	6	0	1	1	1	0	0	1	1	0	0	0	0	1
	7	0	0	0	0	1	0	0	0	0	0	0	0	0
	8	1	1	1	1	1	1	1	1	1	1	1	1	1
G6PDH	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	1	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	1	1	0	0	0	1	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0	1	0
	7	1	1	1	1	1	1	1	1	1	1	1	1	1
LAP	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SKDH	1	1	1	1	1	1	1	1	1	1	1	1	1	1

[a] "0" indicates the absence of a band in a particular position, "1" indicates its presence.

[b] PGI, phosphoglucose isomerase; G6PDH, glucose-6-phosphate dehydrogenase; LAP, leucine aminopeptidase; SKDH, shikimate dehydrogenase.

Table 3.6. [a]Presence or absence of stained protein bands obtained following starch gel electrophoresis of protein extracts from strains of *Chlorellidium* and *Botryochloris.*

[b]Enzyme	Band position	*Chlorellidium*								*Botryochloris*
		Morphotype								
		C1						C2		BC1
		Strain								
		597	757	758	898	G28/7	G31/6	785	871	G19/1
PGI	1	0	0	0	0	0	0	1	0	0
	2	0	0	0	0	0	0	1	0	0
	3	0	0	0	0	0	0	0	0	0
	4	1	1	1	1	1	0	1	0	0
	5	0	0	0	0	0	1	0	1	0
	6	0	0	0	1	0	1	0	0	1
	7	0	0	0	0	0	1	0	0	0
	8	1	1	1	1	1	1	1	1	1
G6PDH	1	0	0	0	0	1	0	0	0	0
	2	1	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	4	0	1	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0
	7	1	1	1	1	1	1	1	1	1
LAP	1	1	1	1	1	1	1	1	1	1
SKDH	1	1	1	1	1	1	1	1	1	1

[a] "0" indicates the absence of a band in a particular position, "1" indicates its presence.

[b] PGI, phosphoglucose isomerase; G6PDH, glucose-6-phosphate dehydrogenase; LAP, leucine amino peptidase and SKDH, shikimate dehydrogenase.

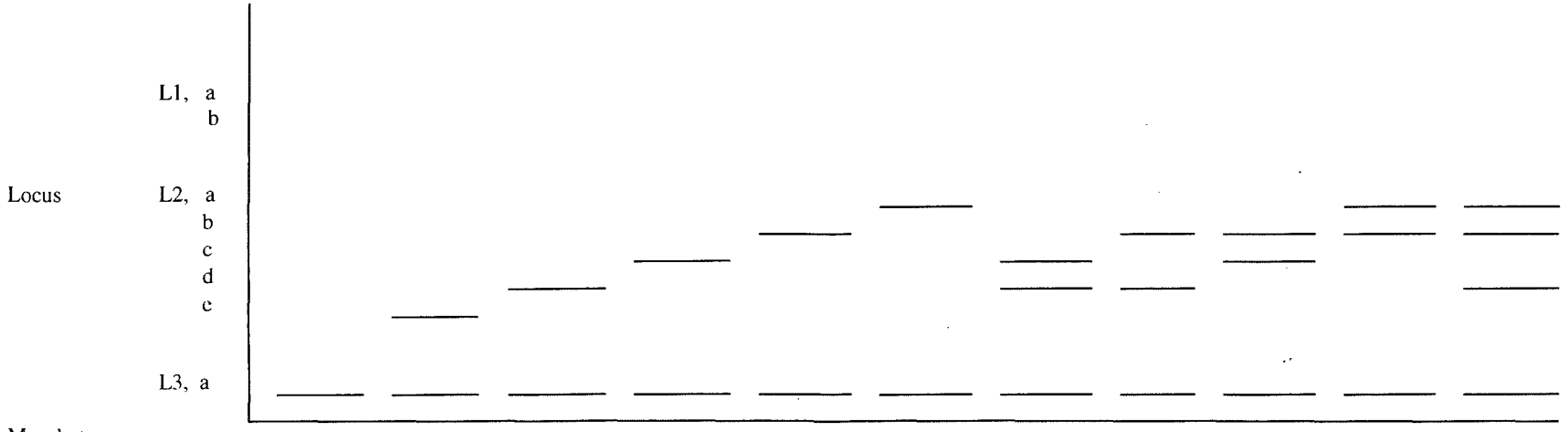
phenotypes (see section 2.4.5). Figs. 34-38 illustrate the banding patterns for each enzyme system surveyed in all strains.

PGI showed the greatest variability of the four isozyme systems (Figs. 34, 36 and 38). Two putative loci (L3 and L2) were recognised for all these genera except five strains from *Botrydiopsis* in which L2 was absent. L3 was common to all strains as just a single band. L1 was absent in *Botrydiopsis* and *Botryochloris* but present in *Chlorellidium*. At L2, five (2a-2e), four (2b-2e) and single (2d) bands were recognised in *Botrydiopsis, Chlorellidium* and *Botryochloris* respectively. Individual strains from *Botrydiopsis* and *Chlorellidium* possessed from one to various combinations of three of these bands.

G6PDH showed three putative loci (L1, L2 and L3; Figs. 35, 37 and 38). L3 was common to all strains as just a single band. L1 was absent in *Botryochloris* and was present in only one strain from *Botrydiopsis* as a single band. Similarly, two bands (1a and 1b) were recognised in two strains from *Chlorellidium* at the same locus and these strains each possessed one of the two bands. L2 was absent in *Botryochloris* but present in eight strains of *Chlorellidium*. In addition, L2 was absent in 20 strains from *Botrydiopsis* but seven strains each possessed one of the four bands (2a-2d) detected at this locus.

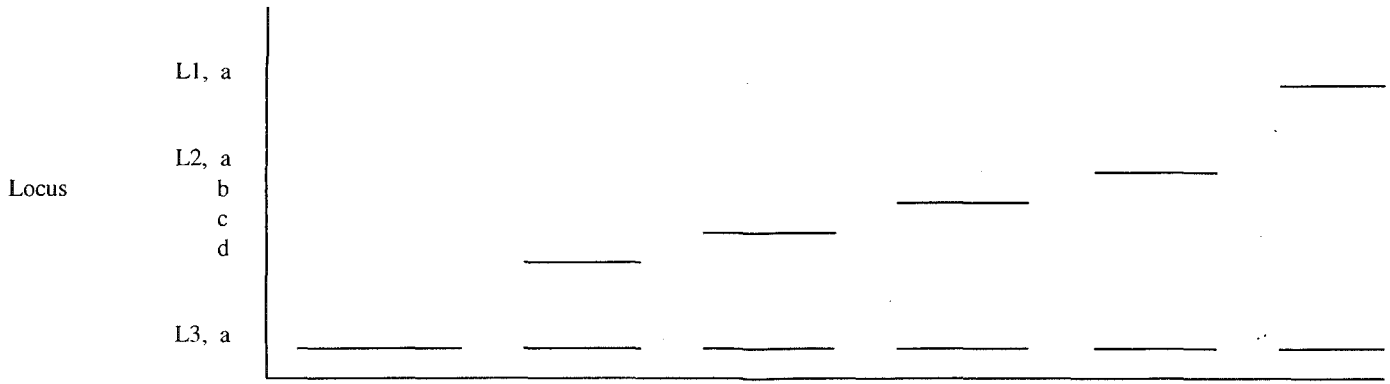
LAP and SKDH each produced a single putative locus L3 for all strains from *Botrydiopsis* and *Chlorellidium* (not shown, similar with Fig. 38), and *Botryochloris* (Fig. 38) as just a single band. Loci L1 and L2 were absent in all three genera. LAP produced a more distinct band than SKDH.

The genetic variation in a population can be expressed as the frequency of polymorphic loci (Skov *et al.,* 1997). The genetic interpretation of the banding pattern of all strains from *Botrydiopsis* and *Chlorellidium* based on putative loci and alleles gave a total of seven and eight loci respectively (Table 3.7). Of these, two loci were polymorphic in *Botrydiopsis* and three in *Chlorellidium* giving a total frequency of polymorphic loci, i.e. ratio of number of polymorphic loci to total number of loci, of 0.29 and 0.43 respectively.



Morphotype											
B1				WI/1 G41/6	801	829 645 894					
B2	G12/1 724 896			897	895			886			
B3										485	
B4			G94/1								
B5				836							
B6	G27/1	G24/8	G99/2 G31/5 837				G9/8				- G19/2
B7				638					877		
B8	864			G27/2							
B9			908								

Fig. 34. The distribution of eleven electrophoretic patterns of the enzyme PGI amongst 27 strains of *Botrydiopsis*. Letters a-e indicate allele number in a particular locus.



Morphotype						
B1	645, 894, 829, 801, G41/6	WI/1				
B2	895, 896, 897, 724, G12/1					886
B3				485		
B4	G94/1					
B5	836					
B6	837, G24/8, G27/1,G31/5		G9/8, G19/2, G99/2			
B7	638				877	
B8	864	G27/2				
B9	908					

Fig. 35. The distribution of six electrophoretic patterns of the enzyme G6PDH amongst 27 strains of *Botrydiopsis*. Letters a-d indicate allele number in a particular locus.

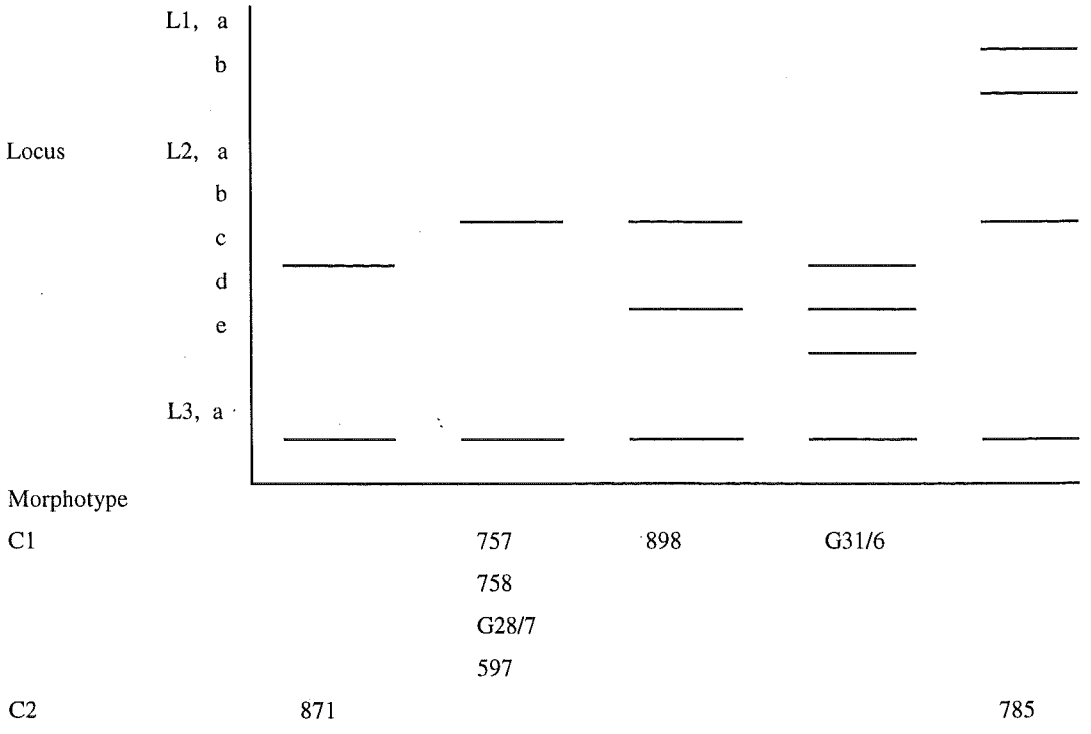


Fig. 36. The distribution of five electrophoretic patterns of the enzyme PGI amongst eight strains of *Chlorellidium.* Letters a-e indicate allele number in a particular locus.

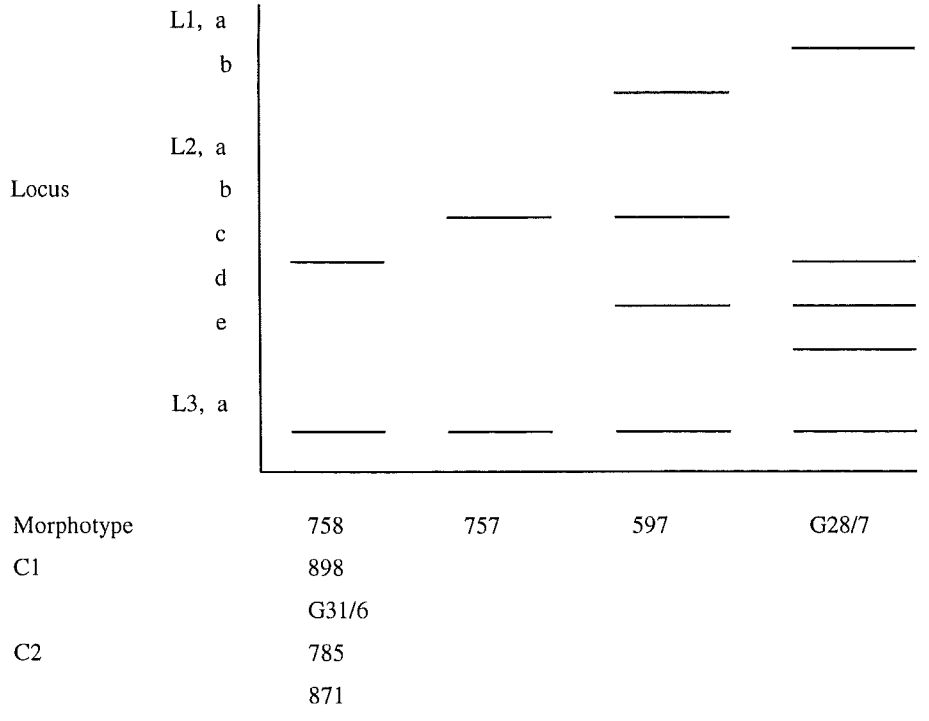


Fig. 37. The distribution of four electrophoretic patterns of the enzyme G6PDH amongst eight strains of *Chlorellidium.* Letters a-e indicate allele number in a particular locus.

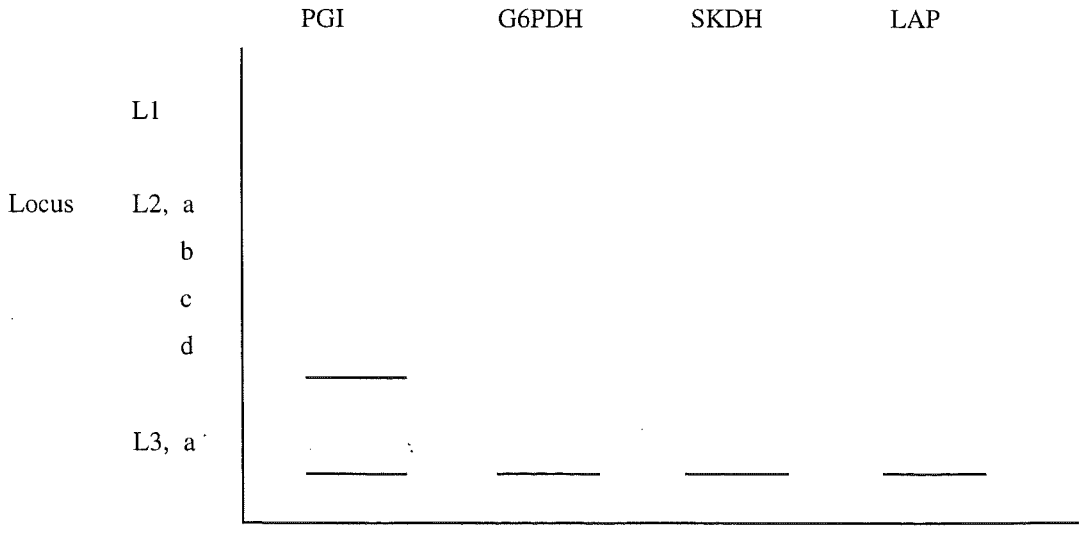


Fig. 38. The distribution of four electrophoretic patterns of the enzymes PGI, G6PDH, SKDH and LAP from single strain of *Botryochloris.* Letters a-d indicate allele number in a particular locus.

Table 3.7. [a]Polymorphism of enzymes of *Botrydiopsis* and *Chlorellidium* detected by electrophoresis.

[b]Enzymes	Loci	No. of alleles *Botrydiopsis*	*Chlorellidium*
PGI	L1	-	2
	L2	5	4
	L3	1	1
G6PDH	L1	1	2
	L2	4	1
	L3	1	1
LAP	L1	-	-
	L2	-	-
	L3	1	1
SKDH	L1	-	-
	L2	-	-
	L3	1	1

[a] Frequency of polymorphic loci = No. of polymorphic loci / Total no. of loci for all enzymes used. For *Botrydiopsis* frequency = 0.29; for *Chlorellidium* frequency = 0.43.

[b] Enzymes: PGI, phosphoglucose isomerase; G6PDH, glucose-6-phosphate dehydrogenase; LAP, leucine amino peptidase; SKDH, shikimate dehydrogenase.

d) Statistical analysis

Combined data sets. Results of UPGMA cluster analysis were expressed as a dendrogram (Fig. 39). There are two main clusters A and B at about the 0.4 dissimilarity level. Strains of both *Botrydiopsis* and *Chlorellidium* are found in both main clusters. Strains of both morphotypes of *Chlorellidium* are in each cluster as are strains of five of the nine morphotypes of *Botrydiopsis*. The single strain of *Botryochloris* clusters with three strains of two morphotypes of *Botrydiopsis* in a subcluster within cluster A.

Data from Botrydiopsis. The dendrogram (Fig. 40) resulting from UPGMA analysis indicates a high level of similarity as the first division is above 0.4 dissimilarity. This is confirmed by S_j values (Table 3.8) from 0.65 to 1.0 for pairwise comparisons amongst all strains. The most apparent feature of the dendrogram is that all strains within particular morphotypes do not cluster together and form separate groups.

Four clusters (A-D; Fig. 40) can be distinguished at the 0.35 dissimilarity level. Strains from Chapman Ridge (485, morphotype B3) and Granite Harbour (G19/2, morphotype B6) form the most dissimilar cluster A. The similarity value of each strain from cluster A compared with each strain from all the remaining clusters ranges from 0.65 to 0.88.

Cluster B, consisting of a single strain (877) of morphotype B7, links with C and D. Two bands (2b and 2c at locus L2) in PGI and a single band (2a at locus L2) in G6PDH separated this strain from the remainder (Fig. 35). The only other strain of this morphotype (638) is mixed with other morphotypes in cluster C. The similarity value (S_j) of these two strains is 0.88.

Clusters C and D contain 18 and six strains respectively. Pairwise comparisons of strains in cluster C compared with strains in cluster D show S_j values from 0.71 to 0.94.

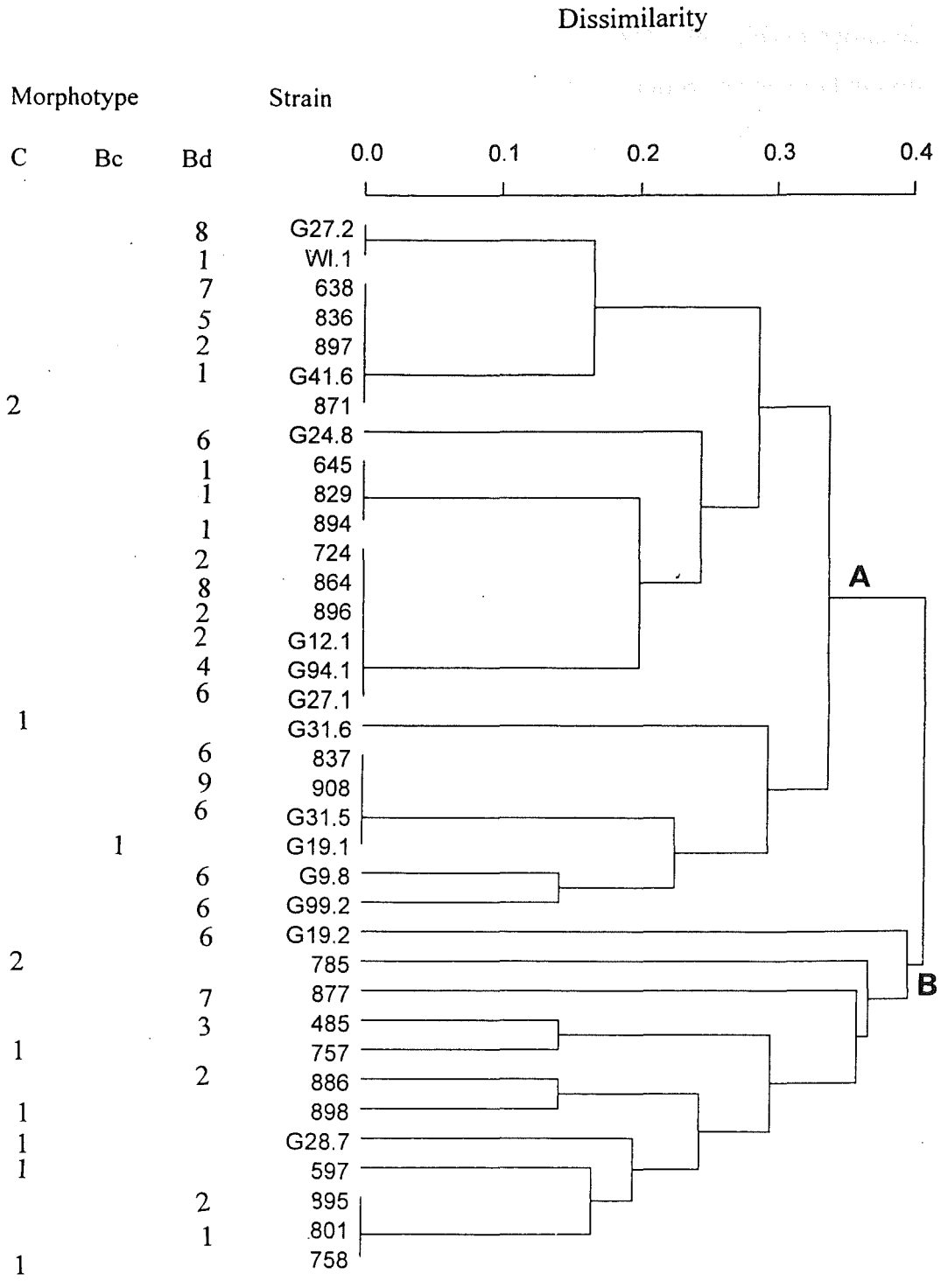


Fig. 39. Cluster analysis dendrogram using similarity coefficient with average linkage based on isozyme patterns of *Botrydiopsis* (Bd), *Botryochloris* (Bc) and *Chlorellidium* (C). A and B are the two major clusters.

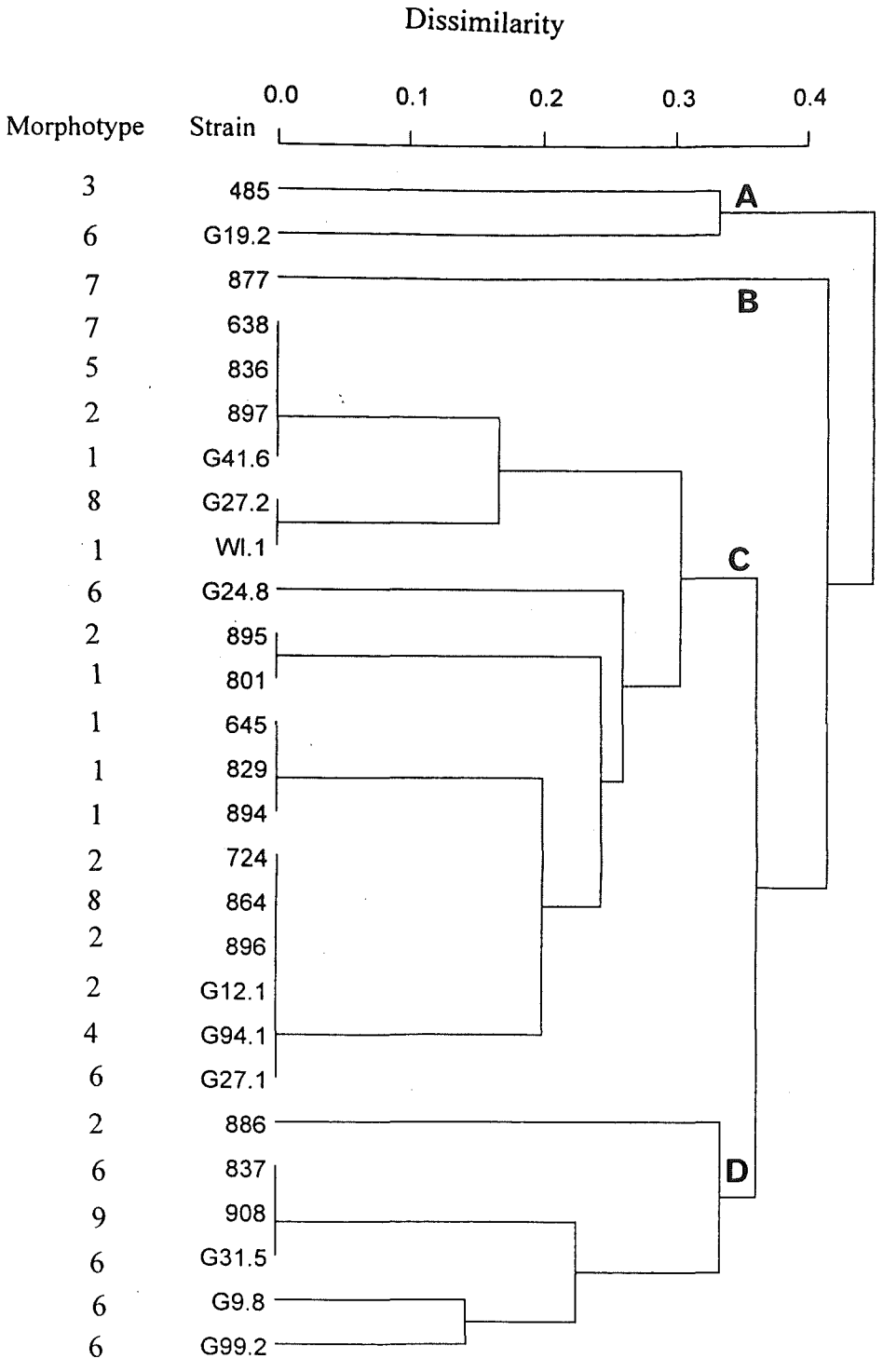


Fig. 40. Cluster analysis dendrogram using similarity coefficient with average linkage based on isozyme patterns of 27 strains of *Botrydiopsis*. A-D are the four major clusters.

Table 3.8. Pairwise comparisons of similarity coefficients (S_j) based on isozyme patterns of 27 strains of *Botrydiopsis.*

Morphotype	Strain	Morphotype	B1					B2						B3	B4	B5	B6							B7		B8		B9
		Strain	645	894	829	801	G41/6	WI/1	895	896	897	886	G12/1	724	485	G94/1	836	837	G9/8	G19/2	G24/8	G27/1	G31/5	G99/2	877	638	864	G27/2	908
B1	645		-	1	1	0.88	0.88	0.82	0.88	0.94	0.88	0.76	0.94	0.94	0.88	0.94	0.88	0.88	0.76	0.82	0.88	0.94	0.88	0.82	0.76	0.88	0.94	0.82	0.88
	894			-	1	0.88	0.88	0.82	0.88	0.94	0.88	0.76	0.94	0.94	0.88	0.94	0.88	0.88	0.76	0.82	0.88	0.94	0.88	0.82	0.76	0.88	0.94	0.82	0.88
	829				-	0.88	0.88	0.82	0.88	0.94	0.88	0.76	0.94	0.94	0.88	0.94	0.88	0.88	0.76	0.82	0.88	0.94	0.88	0.82	0.76	0.88	0.94	0.82	0.88
	801					-	0.88	0.82	1	0.94	0.88	0.88	0.94	0.94	0.88	0.94	0.88	0.88	0.76	0.82	0.88	0.94	0.88	0.82	0.88	0.88	0.94	0.82	0.88
	G41/6						-	0.94	0.88	0.94	1	0.76	0.94	0.94	0.76	0.94	1	0.88	0.88	0.71	0.88	0.94	0.88	0.82	0.88	1	0.94	0.94	0.88
	WI/1							-	0.82	0.88	0.94	0.71	0.88	0.88	0.71	0.88	0.94	0.82	0.82	0.65	0.82	0.88	0.82	0.76	0.82	0.94	0.88	1	0.82
B2	895								-	0.94	0.88	0.88	0.94	0.94	0.88	0.94	0.88	0.88	0.76	0.82	0.88	0.94	0.88	0.82	0.88	0.88	0.94	0.82	0.88
	896									-	0.94	0.82	1	1	0.82	1	0.94	0.94	0.82	0.76	0.94	1	0.94	0.88	0.82	0.94	1	0.88	0.94
	897										-	0.76	0.94	0.94	0.76	0.94	1	0.88	0.88	0.71	0.88	0.94	0.88	0.82	0.88	1	0.94	0.94	0.88
	886											-	0.82	0.82	0.76	0.82	0.76	0.88	0.76	0.82	0.76	0.82	0.88	0.82	0.76	0.76	0.82	0.71	0.88
	G12/1												-	1	0.82	1	0.94	0.94	0.82	0.76	0.94	1	0.94	0.88	0.82	0.94	1	0.88	0.94
	724													-	0.82	1	0.94	0.94	0.82	0.76	0.94	1	0.94	0.88	0.82	0.94	1	0.88	0.94
B3	485														-	0.82	0.76	0.76	0.65	0.82	0.76	0.82	0.76	0.71	0.76	0.76	0.82	0.71	0.76
B4	G94/1															-	0.94	0.94	0.82	0.76	0.94	1	0.94	0.88	0.82	0.94	1	0.88	0.94
B5	836																-	0.88	0.88	0.71	0.88	0.94	0.88	0.82	0.88	1	0.94	0.94	0.88
B6	837																	-	0.88	0.82	0.88	0.94	1	0.94	0.76	0.88	0.94	0.82	1
	G9/8																		-	0.82	0.76	0.82	0.88	0.94	0.76	0.88	0.82	0.82	0.88
	G19/2																			-	0.71	0.76	0.82	0.88	0.71	0.76	0.76	0.65	0.82
	G24/8																				-	0.94	0.88	0.82	0.76	0.88	0.94	0.82	0.88
	G27/1																					-	0.94	0.88	0.82	0.94	1	0.88	0.94
	G31/5																						-	0.94	0.76	0.88	0.94	0.82	1
	G99/2																							-	0.71	0.82	0.88	0.76	0.94
B7	877																								-	0.88	0.82	0.82	0.76
	638																									-	0.94	0.94	0.88
B8	864																										-	0.88	0.94
	G27/2																											-	0.82
B9	908																												-

In addition, there are divisions within clusters A, C and D. Cluster A is further divided into two subclusters based on position of bands in both PGI and G6PDH at locus L2 and the number of bands at L2 in PGI. However, these two subclusters exhibited similar patterns for other enzymes.

Similarly, clusters C and D are further divided into six and four subclusters respectively. The division within cluster C is mostly based on presence of bands at L2 locus in PGI and G6PDH. Likewise, the division within cluster D is based on presence of locus L1 in G6PDH and presence of bands at L2 locus in both PGI and G6PDH.

Data from Chlorellidium. UPGMA analysis resulted in a dendrogram (Fig. 41) in which the most apparent feature is the high degree of similarity of all strains. Their S_j value ranges from 0.65 to 0.94 (Table 3.9). The first division into cluster A and B is above the 0.4 dissimilarity level. These clusters are largely based on the presence in cluster A of band 2c at locus L2 of PGI but its absence in cluster B. Strains of both morphotypes are found in both clusters A and B.

Similarly, clusters C and D are further divided into six and four subclusters respectively. The division within cluster C is mostly based on presence of bands at L2 locus in PGI

Also included in the analysis are six separate clonal isolates of morphotype C1 from a single sample (Granite Harbour sample G28). These all produced identical banding patterns.

3.2.3 Pigment analysis

Results of pigment analysis using HPLC are displayed in Tables 3.10 and 3.11. All strains of *Botrydiopsis* and *Chlorellidium* contain chlorophyll *a* and *c*. There is a total of 45 carotenoids but most of these were present as trace quantities. There are no major

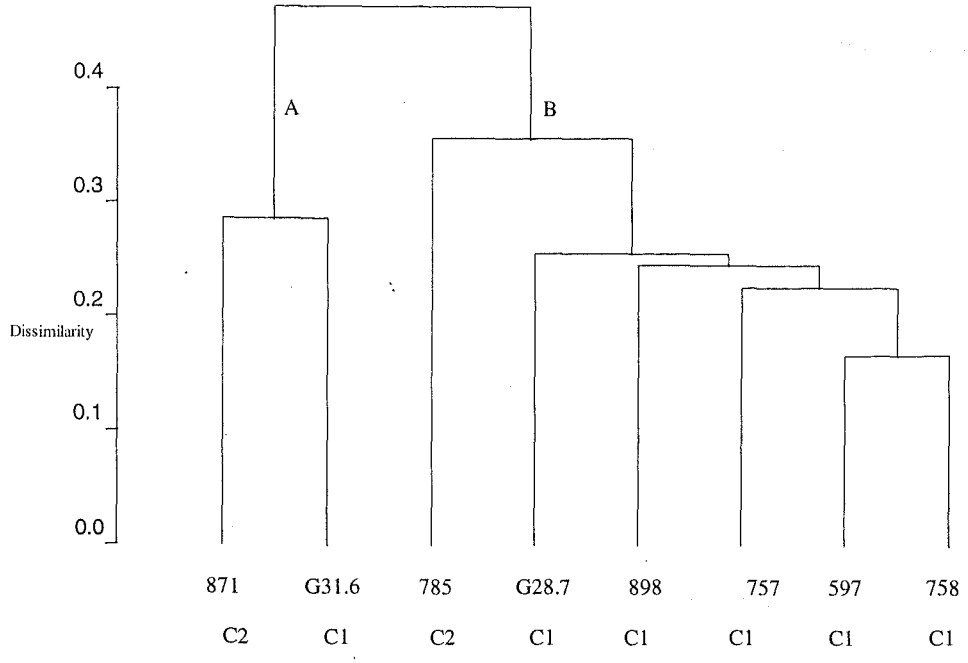


Fig. 41. Cluster analysis dendrogram using similarity coefficient with average linkage based on isozyme patterns of eight strains of *Chlorellidium.* A and B indicate clusters referred to in the text.

Table 3.9. Pairwise comparisons of similarity coefficients (S_j) based on isozyme patterns of eight strains of *Chlorellidium.*

Morphotype	Strain	Morphotype							
		C1						C2	
		Strain							
		597	757	758	898	G28/7	G31/6	785	871
C1	597	-	0.88	0.94	0.88	0.88	0.71	0.82	0.82
	757		-	0.94	0.88	0.88	0.71	0.82	0.82
	758			-	0.94	0.94	0.76	0.88	0.88
	898				-	0.88	0.82	0.82	0.82
	G28/7					-	0.71	0.82	0.82
	G31/6						-	0.65	0.88
C2	785							-	0.76
	871								-

Table. 3.10. [a] Percentage composition of pigments, analysed using HPLC, from 27 strains of *Botrydiopsis.*

Morphotype	Strain no.	Chl *a*	Chl *c*	β-C	Het	Diat	Diad	Vau	9′ cN	tN	Zea	Fux1	Fux2	Fux3	UC1	UC2	UC3	UCR
B1	645	54	16	1	3	3	14	5	t	1	t	1	t	t	t	-	-	1
	894	47	10	2	4	t	16	6	1	2	2	t	t	t	1	1	2	4
	829	53	25	1	2	1	10	4	1	t	-	1	t	t	t	1	-	1
	801	54	9	1	3	2	14	t	1	2	-	-	t	1	8	-	t	2
	G41/6	55	13	1	4	1	15	5	1	2	-	t	t	t	1	1	-	-
	WI/1	55	7	2	4	2	18	6	1	1	-	t	1	1	1	2	-	-
B2	895	56	7	1	4	3	17	4	1	2	-	t	t	t	1	2	-	-
	896	57	9	1	4	3	14	5	1	2	1	t	t	t	1	-	-	1
	897	51	10	1	4	1	11	7	1	2	3	t	1	t	3	2	-	1
	886	59	7	1	4	2	14	6	1	2	t	t	t	t	t	1	-	2
	G12/1	38	7	2	3	3	14	10	1	2	1	-	1	t	4	2	4	8
	724	42	32	1	3	1	10	5	-	t	-	t	2	1	t	2	-	2
B3	485	34	43	1	3	1	9	4	1	t	-	-	t	t	t	1	-	1
B4	G94/1	45	10	1	3	1	15	7	1	3	-	t	1	t	1	2	4	4
B5	836	60	6	1	4	1	17	4	1	1	-	-	1	1	1	3	-	-
B6	837	62	7	2	t	1	13	5	3	1	t	t	t	t	1	2	-	-
	G9/8	46	7	2	5	-	20	6	1	1	4	t	1	1	2	1	t	1
	G19/2	41	8	2	5	1	12	8	2	2	t	t	1	2	1	3	5	7
	G24/8	60	4	1	3	2	18	5	t	1	-	t	t	2	1	1	-	-
	G27/1	47	4	3	5	1	12	5	1	1	-	t	t	4	5	7	-	6
	G31/5	51	7	2	4	2	13	6	1	2	3	-	1	1	3	1	-	3
	G99/2	45	8	1	4	1	17	7	1	2	-	-	1	1	1	2	4	5
B7	877	43	12	1	6	1	19	7	t	2	-	t	t	t	t	2	1	3
	638	54	7	1	7	2	13	4	2	4	-	1	1	t	1	1	t	2
B8	864	51	9	1	5	1	17	3	t	4	-	-	1	3	1	3	-	-
	G27/2	48	9	3	4	1	15	7	1	1	-	t	1	2	3	2	-	3
B9	908	56	6	2	4	1	19	5	1	1	-	t	t	1	1	2	-	-

[a] totals of the percentages may not equal 100 due to rounding errors.
t, indicates pigment was less than 0.5% of the total fraction.
-, indicates the pigment was absent.
Key to pigments: Chl *a*, chlorophyll *a* with degradation products; Chl *c*, chlorophyll *c*; β-C, β-carotene; Het, heteroxanthin; Diat, diatoxanthin; Diad. diadinoxanthin; Vau. vaucherioxanthin; 9′ cN, 9′ cis neoxanthin; tN, trans neoxanthin; Zea, zeaxanthin; Fux1-3, three fucoxanthin-like pigments; UC1-3, three unknown carotenoids; and UCR, remaining unknown carotenoids.

Table. 3.11. Percentage[a] composition of pigments, analysed using HPLC, from eight strains of *Chlorellidium*.

Morphotype	Strain no.	Chl *a*	Chl *c*	β-C	Het	Diat	Diad	Vau	9′ cN	tN	Zea	Fux1	Fux2	Fux3	UC1	UC2	UC3	UCR
C1	597	55	8	1	4	1	15	6	1	2	-	t	1	1	1	2	t	1
	757	48	9	1	4	2	14	7	2	3	-	-	1	1	2	3	-	3
	758	41	10	t	6	3	16	7	1	3	t	t	1	1	1	-	1	9
	898	45	10	2	5	1	16	5	1	2	-	-	t	t	1	2	3	8
	G28/7	40	11	1	5	2	15	7	2	3	-	-	1	1	1	3	1	4
	G31/6	42	9	1	4	-	19	7	t	1	5	-	t	1	1	1	1	5
C2	785	31	14	1	7	1	21	9	1	2	-	t	1	1	1	2	1	5
	871	43	9	1	5	2	16	5	1	3	t	-	1	1	1	3	1	7

[a] totals of the percentages may not equal 100 due to rounding errors.
t, indicates pigment was less than 0.5% of the total fraction.
-, indicates the pigment was absent.
Key to pigments: Chl *a*, chlorophyll *a* with degradation products; Chl *c*, chlorophyll *c*; β-C, β-carotene; Het, heteroxanthin; Diat, diatoxanthin; Diad. diadinoxanthin; Vau. vaucherioxanthin; 9′ cN, 9′ cis neoxanthin; tN, trans neoxanthin; Zea, zeaxanthin; Fux1-3, three fucoxanthin-like pigments; UC1-3, three unknown carotenoids; and UCR, remaining unknown carotenoids.

qualitative and quantitative differences in carotenoid pigments between all strains.

However, there are minor qualitative and quantitative differences of identified carotenoids in all strains. Zeaxanthin is most variable. In *Botrydiopsis*, it varies from absent in 17 strains to 4% in G9/8 and in *Chlorellidium* from absent in five strains to 5% in G 31/6. Diatoxanthin is lacking from *Botrydiopsis* G9/8 and *Chlorellidium* G31/6 but this is interconvertible with diadinoxanthin which both strains possess. 9′ cis neoxanthin is absent from *Botrydiopsis* 724.

Among the 34 unknown carotenoids, only three, UC1, UC2 and UC3 were present at 5% or more in any one strain and these ranged from trace to 8%, 0-7% and 0-5% respectively. None of the remaining 31 unknown carotenoids (UCR, Tables 3.10, 3.11) exceeded 3% in any one strain.

3.3 Chlorophyta

The phylum Chlorophyta consists of a natural group and it is well differentiated from all other groups of algae (Hoek *et al.*, 1995). It includes many unicellular, colonial and multicellular forms. Nuclear division (mitosis) and cell division (cytokinesis), architecture of flagellate cells, level of organization, cell wall and pigment composition, storage products and type of life-history provide the most important characters for distinguishing the classes within Chlorophyta. Hoek *et al.* (1995) subdivide this phylum into eleven classes and 22 orders and their system is followed below. The taxonomic compilation of Ettl and Gärtner (1995) is used here for identification to generic and specific level.

The present investigation has concentrated on species which have been poorly described and which are previously unknown from Antarctica. The following provides detailed descriptions of morphotypes using light microscopy. In addition, TEM was utilised for *Stichococcus*. Generic characteristics are described first. These are translations of descriptions provided by Ettl and Gärtner (1995). They are followed by descriptions of

characteristic features of each morphotype isolated from Antarctic samples.

Class: Chlamydophyceae

Order: Chlamydomonadales

Family: Chlamydomonadaceae

***Chlamydomonas* Ehrenberg 1833**

Cells single, free-living, motile, oval, ellipsoidal, globular or fusiform, with ellipsoidal stigma, with or without papillae, from which two equal flagella arise, in transverse section spherical or slightly ellipsoidal. Chloroplast of variable form containing one to several pyrenoids. One, two or several contractile vacuoles. Reproduction by aplanospores, zoospores and gametes. Zoospores mostly two to eight, occasionally several. Palmella stage frequent. Sexual reproduction isogamous or oogamous, occasionally zygote with sculptured wall.

Chlamydomonas morphotype 1 (C. cf. intermedia Chodat)
(Figs. 42.1-12 and 43.1-4)

Cells ellipsoidal, cylindrical to spindle-shaped with narrow apices (Figs. 42.1-3 and 43.1), occasionally spherical, 7-15 by 4-8.5 μm. Apical papillae flat or hump-like (Fig. 43.2). Flagella as long as cell body. Contractile vacuoles two, anterior. Nucleus single, posterior. Chloroplast parietal, band-shaped, appears cup-like in apical view (Fig. 42.7). Pyrenoid spherical to ellipsoidal, up to 3.5 μm diameter, situated in the basal thickened part of the chloroplast. Starch sheath entire, perforated and lobed (Fig. 42.12). Stigma dark brown, lying anterior to pyrenoid (Figs. 42.1-3).

Sporangia up to 15 by 8.5 μm, containing 2-4 spores (Figs. 42.9-10). Spores released by rupture of sporangium wall. Aplanospores spherical to ellipsoidal, 3.5-10 μm diameter (Fig. 42.11). Zoospores ellipsoidal, spherical to spindle-shaped with narrow anterior and hyaline posterior, 6.5-12.5 by 3-7.5 μm (Figs. 42.4-8 and 43.3-4).

Distribution: Recorded from Scott Base.

Chlamydomonas morphotype 2 (unidentified species of Chlamydomonas)
(Figs. 42.13-22 and 43.5-6)

Cells ellipsoidal to cylindrical, occasionally with narrow posterior end, 11-20 by 7.5-15.5 μm (Figs. 42.13-14, 17). Apical papillae two, hump-like. Flagella up to 12.5 μm long. Contractile vacuoles two, anterior. Nucleus single, median to posterior. Chloroplast extensive with perforations, lobed (Figs. 42.15 and 43.5-6). Pyrenoid spherical to ellipsoidal, up to 4.5 μm diameter, situated at thickened part of chloroplast. Starch sheath entire, perforated and lobed (Figs. 42.19, 22). Stigma dark brown, lying anterior to pyrenoid.

Sporangia up to 23.5 μm diameter, containing 2-8 spores (Figs. 42.16). Spores released by rupture of sporangium wall (Fig. 42.20). Aplanospores mostly ellipsoidal, occasionally spherical, 6-12.5 by 6.5-9.5 μm (Fig. 42.21). Zoospores ellipsoidal to cylindrical, 9-16.5 by 6-13 μm (Fig. 42.18). Palmella stage and sexual reproduction not found.

Distribution: Recorded from Castle Rock and Scott Base.

Order: Chlorococcales
Family: Chlorococcaceae
***Chlorococcum* Meneghini 1842**

Cells solitary or in temporary aggregations, never embedded in colonial mucilage, ellipsoidal to spherical, uninucleate or multinucleate. Cell wall smooth, thin, sometimes thickening with age. Chloroplast a parietal hollow sphere, with or without unilateral opening, with one to many pyrenoids. Asexual reproduction by autospores or zoospores. Zoospores equally biflagellate, walled. Sexual reproduction by fusion of motile, biflagellate, walled gametes.

Chlorococcum (Meneghini) Starr includes cells with contractile vacuoles (Starr, 1955).

Fig. 42. *Chlamydomonas* and *Chlorococcum.*

1-12. *Chlamydomonas* morphotype 1. 1-3, variability of vegetative cells with band-shaped chloroplasts with pyrenoids. 1, cylindrical mature cell with a hump-like papilla. 2, ellipsoidal mature cell with flat papilla. 3, spindle mature cell with narrow anterior and hyaline posterior. 4-8, ellipsoidal to spindle zoospores with flat or hump-like papilla, posterior nucleus, anterior stigma and two equal flagella. 7, chloroplast cup-like in apical view. 8, zoospore following loss of flagella. 9-10, sporangia containing two spores. 11, spherical aplanospore. 12, pyrenoids with entire, perforated and lobed starch sheaths.

13-22. *Chlamydomonas* morphotype 2. 13-14, 17, ellipsoidal to cylindrical mature cells with two hump-like papillae and a posterior nucleus. 15, chloroplast with perforations. 16, aplanosporangium containing eight aplanospores. 18, ellipsoidal zoospores with two hump-like papillae, a posterior nucleus and two equal flagella. 19, pyrenoids with entire, lobed and perforated starch sheaths. 20, spores released by rupture of sporangium wall. 21, ellipsoidal aplanospore. 22, chloroplast lobed and pyrenoid at thickened part of chloroplast.

23-33. *Chlorococcum* morphotype 4. 23-26, 28, spherical to ellipsoidal cells with cup-shaped chloroplasts with one to two pyrenoids. 27, pyrenoids with starch granules. 29, ellipsoidal to fusiform zoospores with flat or two hump-like papillae, anterior nucleus, median to posterior stigma and two equal flagella. 30, spherical autospore. 31, sporangium with two spores. 32, zoosporangium containing four zoospores. 33, spores released by rupture of sporangium wall.

Scale bar is 10 μm.

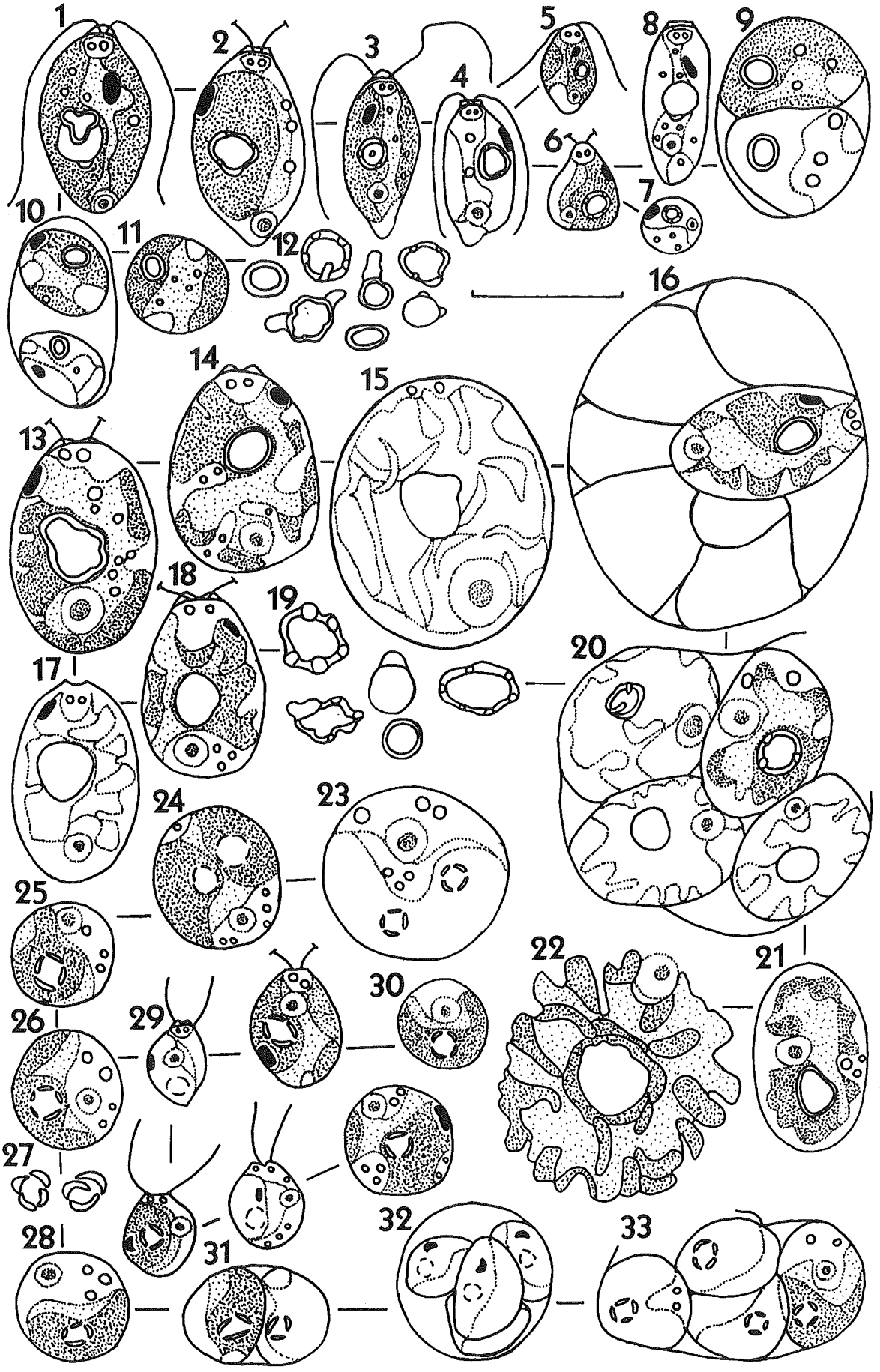

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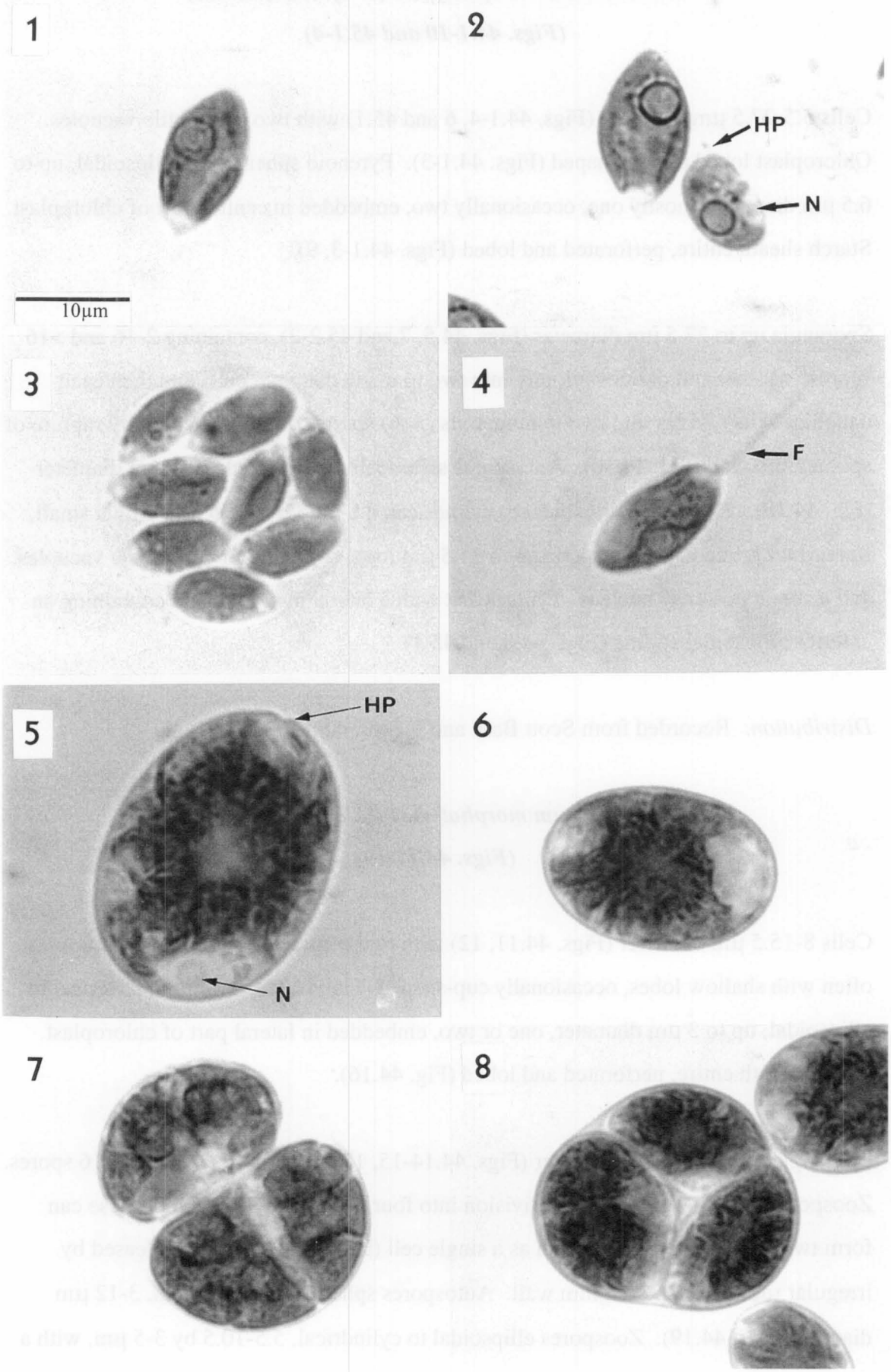
Fig. 43. ***Chlamydomonas,*** **LM.**

1-4. Morphotype 1. 1, Adult cell with hyaline posterior end and band-shaped chloroplast. 2, two cells, one cell showing posterior nucleus (N) and hump-like papilla (HP). 3, zoosporangium containing eight zoospores. 4, ellipsoidal zoospore with flagellum (F) and posterior nucleus.

5-8. Morphotype 2. 5, cell with hump-like papilla (HP) and posterior nucleus (N). 6, adult cell. 7, sporangium. 8, aplanosporangium contains four aplanospores.

The scale in 1 also applies to 2-8.


1
10µm
2
HP
N
3
4
F
5
HP
N
6
7
8

Chlorococcum morphotype 1 (C. cf. elkhartiense)
(Figs. 44.1-10 and 45.1-4)

Cells 5.5-27.5 μm diameter (Figs. 44.1-4, 6 and 45.1) with two contractile vacuoles. Chloroplast lobed to cup-shaped (Figs. 44.1-3). Pyrenoid spherical to ellipsoidal, up to 6.5 μm diameter, mostly one, occasionally two, embedded in central part of chloroplast. Starch sheath entire, perforated and lobed (Figs. 44.1-3, 9).

Sporangia up to 27.5 μm diameter (Figs. 44.5, 7 and 45.2-3), containing 2-16 and >16 spores. Mother cell divides initially into two to many daughter cells and then each daughter cells divides into two to numerous (>16) spores. Spores released by rupture of sporangium wall (Fig. 44.10). Autospores spherical to ellipsoidal, 3-15 μm diameter (Fig. 44.10). Zoospores ellipsoidal to cylindrical, 4.5-12.5 by 3-6.5 μm, with a small, flat or hump-like papilla, flagella up to 11.5 μm long, two anterior contractile vacuoles, and a single posterior nucleus. Chloroplast with a lateral pyrenoid, and containing an anterior ellipsoidal stigma (Figs. 44.8 and 45.4).

Distribution: Recorded from Scott Base and Inexpressible Island.

Chlorococcum morphotype 2 (C. cf. infusionum)
(Figs. 44.11-19)

Cells 8-15.5 μm diameter (Figs. 44.11, 12) with two contractile vacuoles. Chloroplast often with shallow lobes, occasionally cup-shaped (Fig. 44.13). Pyrenoid spherical to ellipsoidal, up to 3 μm diameter, one or two, embedded in lateral part of chloroplast. Starch sheath entire, perforated and lobed (Fig. 44.16).

Sporangia up to 17.5 μm diameter (Figs. 44.14-15, 17), containing 2-16 and >16 spores. Zoosporangia undergo a primary division into four daughter cells, each of these can form two or four spores or remain as a single cell (Fig. 44.15). Spores released by irregular rupture of sporangium wall. Autospores spherical to ellipsoidal, 3-12 μm diameter (Fig. 44.19). Zoospores ellipsoidal to cylindrical, 5.5-10.5 by 3-5 μm, with a

small, flat or hump-like papilla, flagella up to 9 μm long, two anterior contractile vacuoles and a single posterior nucleus. Chloroplast with a lateral pyrenoid, and containing an anterior ellipsoidal stigma (Fig. 44.18).

Distribution: Recorded from Scott Base.

Chlorococcum morphotype 3 (C. tatrense Archibald)
(Figs. 44.20-28 and 45.5-7)

Cells 7.5-25 μm diameter (Figs. 44.20, 23-24 and 45.5) with two contractile vacuoles. Chloroplast cup-shaped to lobed (Figs. 44.20, 23-24), stripe-like in surface view (Fig. 44.22). Pyrenoid spherical to ellipsoidal, up to 3 μm diameter, embedded in lateral part of chloroplast. Starch sheath entire, perforated and lobed (Fig. 44.21).

Sporangia up to 14 μm diameter (Figs. 44.25, 26 and 45.6), containing 2-8 spores. Spores released by irregular rupture of sporangium wall. Autospores spherical to ellipsoidal, 4-10 μm diameter (Fig. 44.28). Zoospores cylindrical to ellipsoidal, 6.5-9 by 3.5-6 μm, with a small, flat or hump-like papillae, flagella up to 10 μm long, two anterior contractile vacuoles, and a single posterior nucleus. Chloroplast with a lateral pyrenoid, containing an anterior ellipsoidal stigma (Figs. 44.27 and 45.6).

Distribution: Recorded from Victoria Valley.

Chlorococcum morphotype 4 (Chlorococcum sp. A)
(Figs. 42.23-33 and 45.8)

Cells 5-17 μm diameter, uninucleate (Figs. 42.23-25, 28). Chloroplast cup-shaped (Figs. 42.23-25, 28). Pyrenoid spherical to ellipsoidal, up to 3 μm diameter, mostly one occasionally two, embedded in lateral thick region of chloroplast. Starch granules 2 to 5 (Fig. 42.27).

Sporangia up to 12.5 μm diameter (Figs. 42.31-32), containing 2-6 spores. Spores

Fig. 44. ***Chlorococcum.***

1-10. Morphotype 1. 1-3, 6, spherical to ellipsoidal cells with cup-shaped to lobed chloroplasts with single pyrenoid and with two contractile vacuoles. 4, loosely clustered cells. 5, zoosporangium with a distinct stigma in each chloroplast. 7, autosporangium containing six autospores. 8, zoospores with small, flat or a hump-like papilla, posterior nucleus, anterior stigma and two equal flagella. 9, pyrenoids with entire, perforated and lobed starch sheaths. 10, spores released by rupture of sporangium wall.

11-19. Morphotype 2. 11, 12, spherical to ellipsoidal cells with shallow lobed to cup-shaped chloroplasts with single pyrenoid and with two contractile vacuoles. 13, chloroplast stripe-like in surface view. 14, autosporangium containing six autospores. 15, zoosporangium with four daughter cells, two of them each formed two spores, one with four spores and one remained as single. 16, pyrenoids with entire, perforated and lobed starch sheaths. 17, zoosporangium with four daughter cells having two to four zoospores and with a distinct stigma in each chloroplast. 18, zoospores with small, flat or a hump-like papilla, posterior nucleus, anterior stigma and two equal flagella. 19, ellipsoidal autospore.

20-28. Morphotype 3. 20, 23-24, spherical to ellipsoidal cells with cup-shaped to lobed chloroplast with single pyrenoid and with two contractile vacuoles. 21, pyrenoids with entire, perforated and lobed starch sheaths. 22, chloroplast stripe-like in surface view. 25, zoosporangium containing four zoospores with a distinct stigma in each chloroplast. 26, autosporangium containing six autospores. 27, zoospores with small, flat or a hump-like papilla, posterior nucleus, anterior stigma and two equal flagella. 28, autospore with single chloroplast.

Scale bar is 10 μm.

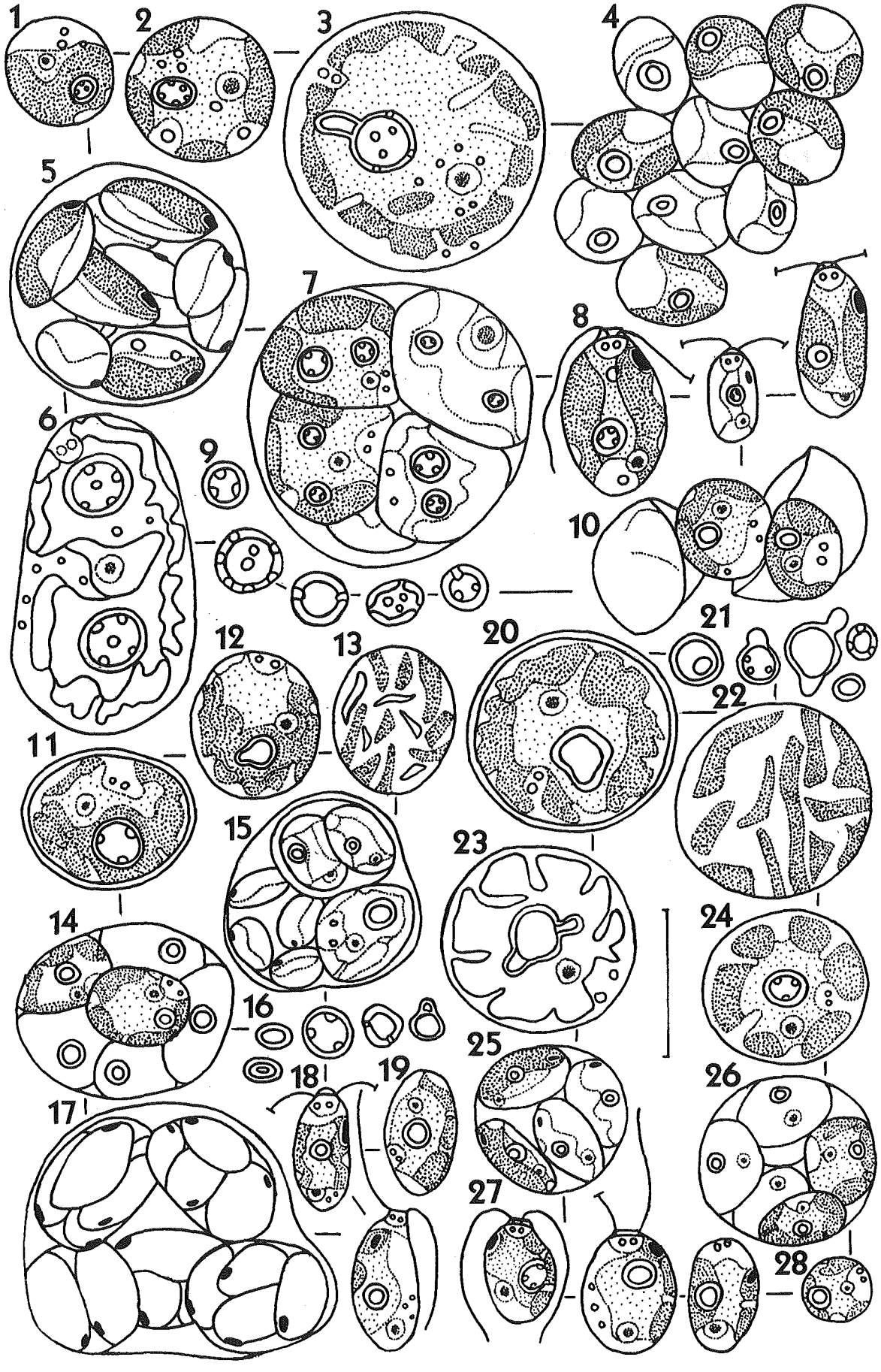

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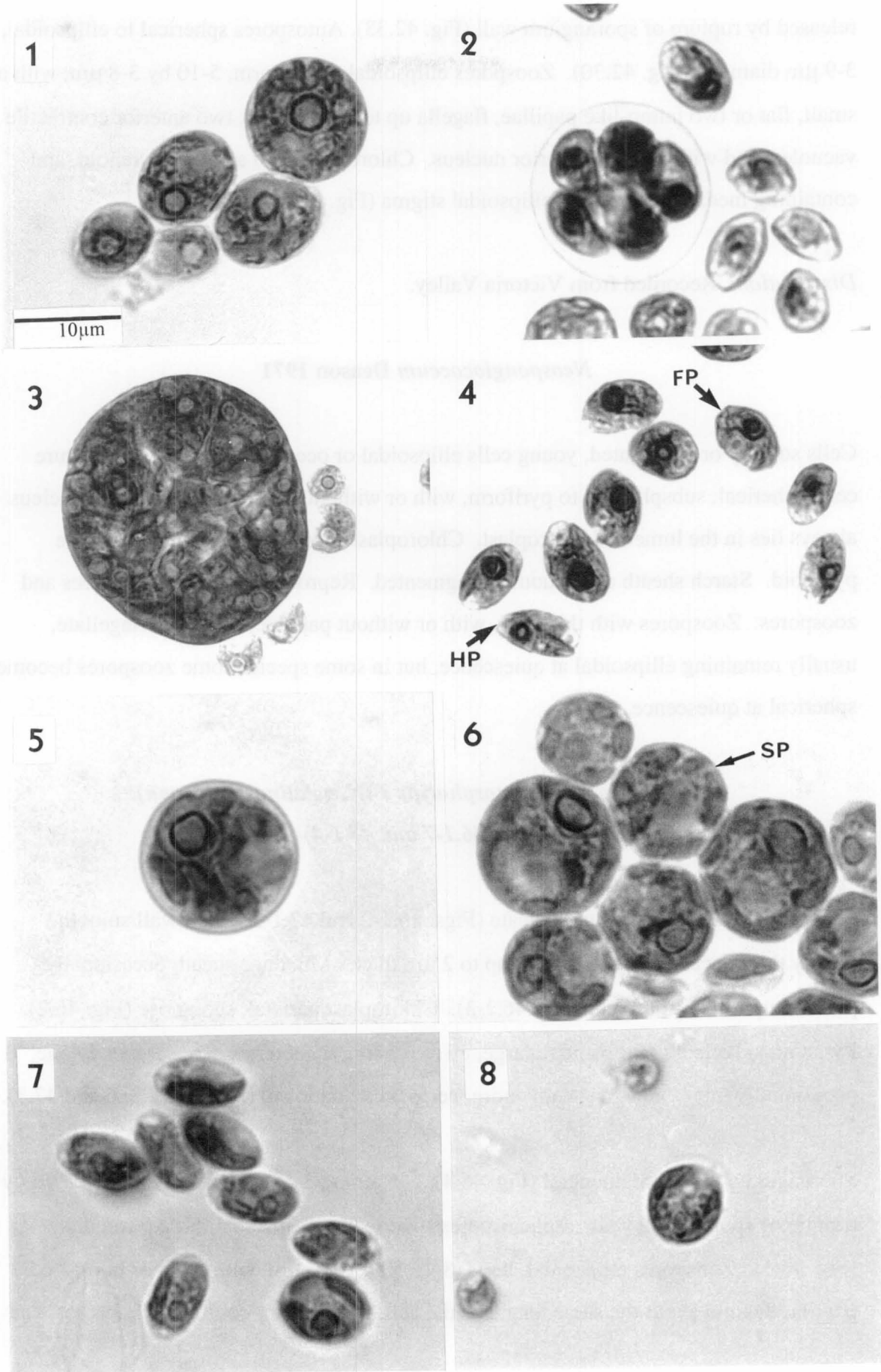
Fig. 45. *Chlorococcum,* LM.

1-4. Morphotype 1. 1, adult cells each with lobed chloroplast and pyrenoid. 2, sporangium and zoospores, sporangium containing six spores. 3, large autosporangium containing numerous autospores. 4, zoospores each with flat (FP) or hump-like papilla (HP), distinct pyrenoid and posterior nucleus.

5-7. Morphotype 3. 5, single cell with lobed chloroplast and with a pyrenoid. 6, group of vegetative cells, single cell transforming into sporangium (SP). 7, zoospores each with distinct pyrenoid.

8. Morphotype 4. 8, young vegetative cell with cup-shaped chloroplast.

Scale in 1 also applies to 2-8.


1
10μm
2
3
4
FP
HP
5
6
SP
7
8

released by rupture of sporangium wall (Fig. 42.33) Autospores spherical to ellipsoidal, 3-9 μm diameter (Fig. 42.30). Zoospores ellipsoidal to fusiform, 5-10 by 3-8 μm, with a small, flat or two hump-like papillae, flagella up to 10 μm long, two anterior contractile vacuoles, and with a single anterior nucleus. Chloroplast with a lateral pyrenoid, and containing median to posterior ellipsoidal stigma (Fig. 42.29).

Distribution: Recorded from Victoria Valley.

Neospongiococcum Deason 1971

Cells solitary or aggregated, young cells ellipsoidal or occasionally spherical, mature cells spherical, subspherical to pyriform, with or without contractile vacuoles. Nucleus always lies in the lumen of chloroplast. Chloroplast spongiose, having at least one pyrenoid. Starch sheath continuous or segmented. Reproduction by aplanospores and zoospores. Zoospores with thin wall, with or without papillae, equally biflagellate, usually remaining ellipsoidal at quiescence, but in some species some zoospores become spherical at quiescence.

Neospongiococcum morphotype 1 (N. gelatinosum forma)
(Figs. 46.1-7 and 47.1-4)

Cells 10-31 μm diameter, uninucleate (Figs. 46.1-2 and 47.1-2). Cell wall smooth, mostly thin, occasionally stratified, up to 2 μm thick. Mucilage sheath occasionally present, up to 2.5 μm thick (Figs. 46.1-3). Chloroplast parietal, spongiose (Fig. 46.2). Pyrenoid spherical to ellipsoidal, large, up to 6.5 μm diameter, excentric, mostly one, occasionally three. Starch sheath entire, perforated and lobed (Figs. 46.1-3, 6 and 47.3).

Sporangia up to 31 μm diameter (Fig. 46.4), containing 2-16 spores. Spores released by rupture of sporangium wall. Aplanospores spherical to ellipsoidal, 6-12.5 μm diameter (Fig. 46.5). Zoospores ellipsoidal, large, 8-12.5 by 4-7.5 μm, with a flat or hump-like papilla, flagella about the same length as the cell, two anterior contractile vacuoles, and

a single posterior nucleus. Chloroplast with a lateral pyrenoid, and containing an anterior ellipsoidal stigma (Figs. 46.7 and 47.4).

Distribution: Recorded from Scott Base.

Radiosphaera Snow ex Herndon 1958

Cells solitary, ellipsoidal to spherical, uninucleate. Chloroplast in young cell 'H'-shaped, in older cells asteroid or lobed with deeply incised margin, having one to two pyrenoids with starch. One species has two large contractile vacuoles. Reproduction by aplanospores and zoospores. Zoospores ellipsoidal with thin wall, and two equal flagella about the same length as the cell, papilla simple to bi-lobed.

Radiosphaera morphotype 1 (Radipsphaera sp. A)
(Figs. 46.8-15 and 47.5-8)

Cells mostly solitary, occasionally forming loose aggregates (Figs. 46.8-9), 10-36 μm diameter. Contractile vacuoles two, opposite to nucleus. Cell wall smooth, thin. Chloroplast axial, asteroidal, with forked lobes extending from central portion to cell wall and which flatten against wall and appear as polygonal plates in surface view (Figs. 46.10 and 47.5-6). Pyrenoid spherical to ellipsoidal, 6 μm diameter, embedded in central portion of chloroplast. Starch sheath entire, perforated and lobed (Fig. 46.12).

Sporangia (Figs. 46.11, 15 and 47.7-8), up to 17.5 μm diameter, containing 2-32 spores, 4 aplanospores frequent. Spores released by dissolution of sporangium wall. Aplanospores ellipsoidal to spherical, 5-13 μm diameter (Fig. 46.14). Zoospores ellipsoidal, 8-13 by 6-8.5 μm, with two hump-like papillae, a single axial chloroplast with a central pyrenoid, median to anterior ellipsoidal stigma, two anterior contractile vacuoles, and a single median nucleus (Fig. 46.13).

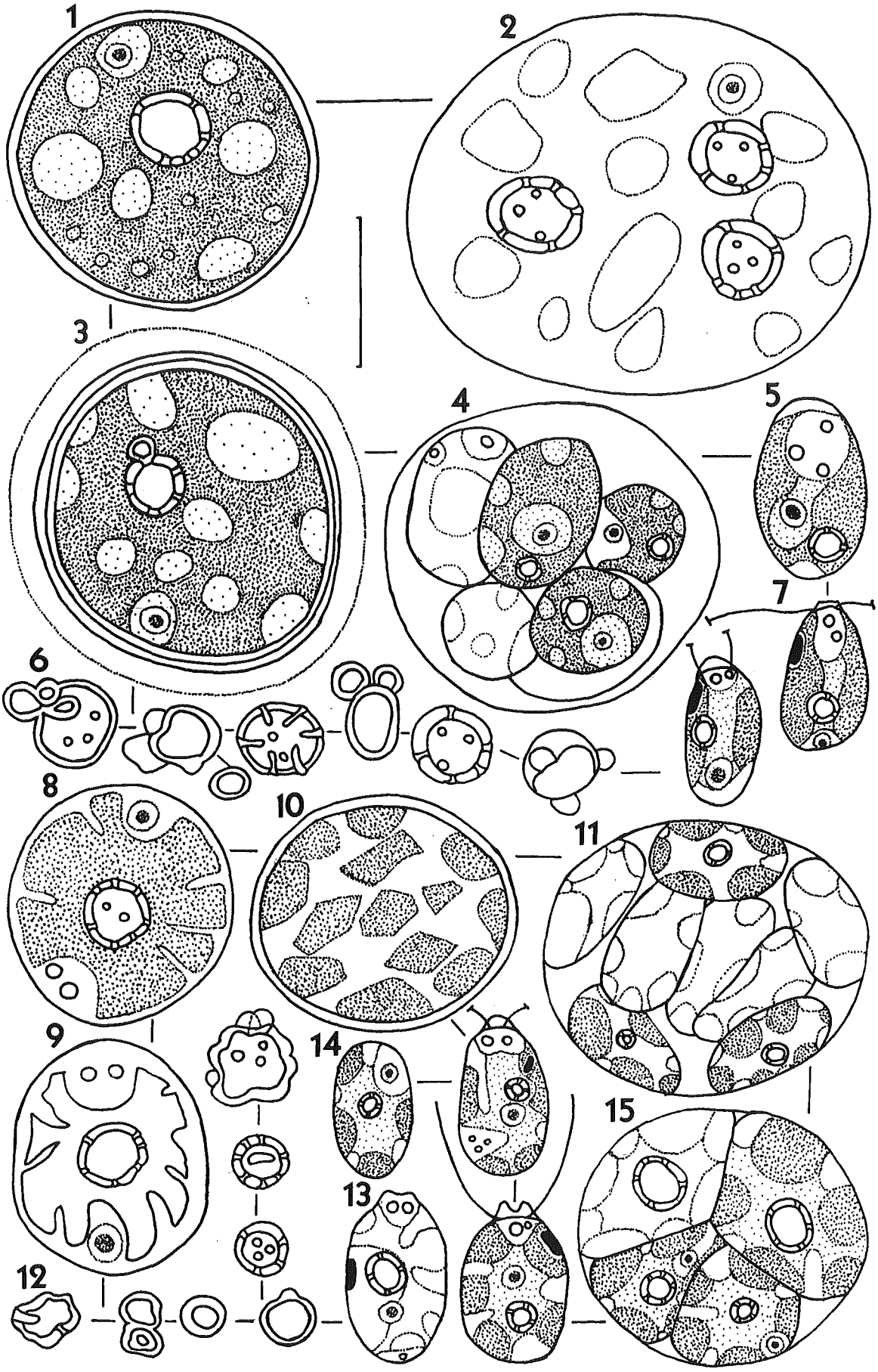
Distribution: Recorded from Victoria Valley.

Fig. 46. ***Neospongiococcum*** **and** ***Radiosphaera.***

1-7. *Neospongiococcum* morphotype 1. 1-2, spherical to ellipsoidal, uninucleate cells with spongiose chloroplasts with one to three pyrenoids. 3, adult cell with stratified wall and mucilage sheath. 4, aplanosporangium containing six aplanospores. 5, ellipsoidal aplanospore. 6, pyrenoids with entire, perforated and lobed starch sheaths. 7, zoospores with flat or a hump-like papilla, posterior nucleus, anterior stigma and two equal flagella.

8-15. *Radiosphaera* morphotype 1. 8-9, spherical to ellipsoidal, uninucleate cells with asteroidal chloroplasts with single pyrenoid and with two contractile vacuoles. 10, surface view showing polygonal ends of the radiating lobes of the chloroplast where these meet the cell wall. 11, sporangium containing eight spores. 12, pyrenoids with entire, perforated and lobed starch sheaths. 13, zoospores with two hump-like papillae, median nucleus, median to anterior stigma and two equal flagella. 14, ellipsoidal aplanospore. 15, aplanosporangium containing four aplanospores.

Scale bar is 10 μm.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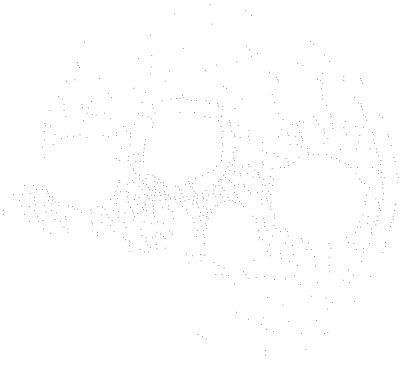
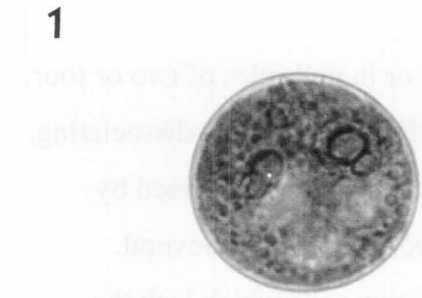


Fig. 47. *Neospongiococcum* and *Radiosphaera,* LM.

1-4. *Neospongiococcum* morphotype 1. 1, cell with spongiose chloroplast. 2, cell showing two pyrenoids each with entire starch sheath. 3, group of adult cells showing pyrenoid each with lobed starch sheath (SS). 4, zoospores following loss of flagella.

5-8. *Radiosphaera* morphotype 1. 5, cell with asteroidal chloroplast. 6, cell with pyrenoid (P) and with perforated starch sheath. 7, aplanosporangium containing eight aplanospores. 8, aplanosporangium with four aplanospores.

Scale in 1 also applies to 2-8.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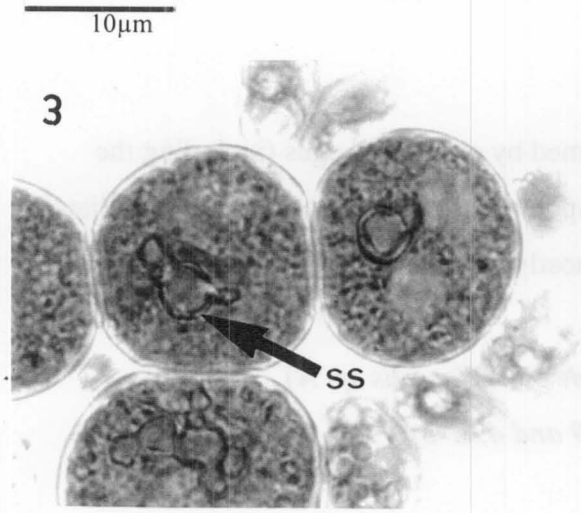
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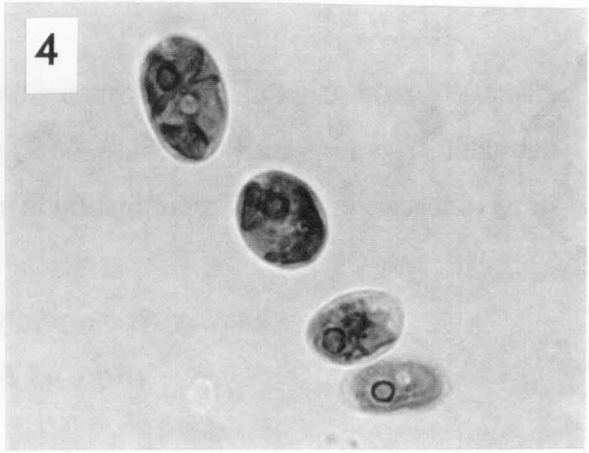
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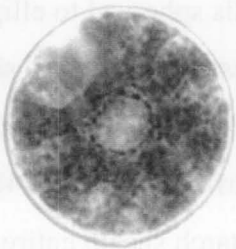
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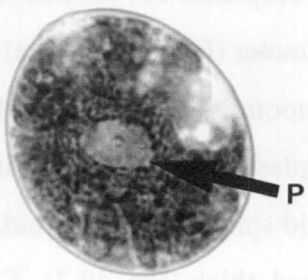
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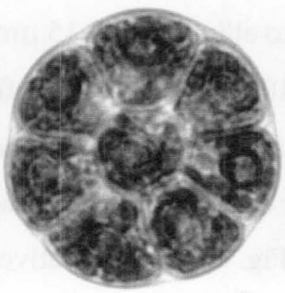
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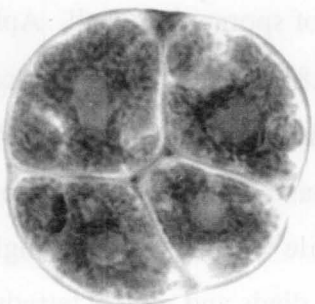
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Tetracystis Brown and Bold 1964

Vegetative cells isolated or in groups of two, four, eight, or in multiples of two or four, the daughter cells of the groups associated at first, sometimes secondarily dissociating. Cells with a hollow, more or less massive, parietal chloroplast, often traversed by fissures, with a pyrenoid. Contractile vacuoles one to two, occasionally several. Asexual reproduction by division into two to eight vegetative cells which lack the potentiality of direct motility, thus differing from aplanospores which arise from motile or potentially motile precursors.

Asexual reproduction also by zoospores formed by cells of all ages (including the daughter vegetative cells of tetrad), the zoospores not becoming spherical immediately upon quiescence. Sexual reproduction by mostly isogamy, occasionally anisogamy.

Tetracystis morphotype 1 (Tetracystis sp. A)
(Figs. 48.1-9 and 49.1-3)

Cells in groups of two or four, many tetrads loosely associated by mucilage sheath but tetrad complexes not formed (Figs. 48.3 and 49.2-3). Cells spherical to ellipsoidal, 8-18 μm diameter (Figs. 48.1-2 and 49.1). Contractile vacuoles two. Nucleus single. Cell wall smooth, thin. Mucilage sheath thin, occasionally up to 2 μm in diameter. Chloroplast parietal, lobed, with fissures, stripe-like in surface view (Fig. 48.4). Pyrenoid spherical to ellipsoidal, up to 6 μm diameter. Starch sheath entire, perforated and lobed, thick (Fig. 48.7). Carotenoid accumulation in old culture.

Sporangia up to 22.5 μm diameter, containing 2-8 spores (Fig. 48.5). Spores released by rupture of sporangium wall. Aplanospores spherical to ellipsoidal, 5-15 μm diameter (Fig. 48.8). Zoospores cylindrical to ellipsoidal, 3.5-10.5 by 3-8.5, with a flat or hump-like papilla, two equal flagella about the same length as the cell, single parietal chloroplast with a lateral pyrenoid, median to anterior ellipsoidal stigma, two anterior contractile vacuoles, with a single posterior nucleus (Fig. 48.9). Vegetative division forming diads and mostly tetrads of daughter cells closely associated with parental cell wall (Fig. 48.6).

Distribution: Recorded from Marie Byrd Land, Scott Base and Granite Harbour.

Tetracystis morphotype 2 (Tetracystis sp. A forma)
(Figs. 48.10-21 and Figs. 49.4-8)

Cells mostly in tetrads, few diads, tetrads frequently aggregate to form complexes (Figs. 48.15-16 and 49.5-8). Cells spherical to ellipsoidal (Figs. 48.10-12 and 49.4), 9-23 µm diameter. Contractile vacuoles two in young cell but absent in mature cell. Nucleus single. Cell wall smooth, thin, occasionally stratified, up to 2 µm thick. Mucilage sheath thin, occasionally up to 2 µm thick (Fig. 48.21). Chloroplast parietal, lobed, cup-shaped, with fissured margin, stripe-like in surface view (Fig. 48.13). Pyrenoid spherical to ellipsoidal, up to 6 µm diameter. Starch sheath entire, perforated and lobed (Fig. 48.20). Carotenoid accumulation in old culture.

Sporangia up to 32 µm diameter, containing 2-24 or >24 spores (Figs. 48.17 and 49.6-7). Spores released by rupture of sporangium wall. Aplanospores spherical to ellipsoidal, 5-14 µm diameter (Fig. 48.18). Zoospores spherical, ellipsoidal to fusiform, 5-10 by 2.5-7, and with a flat or two indistinct hump-like papillae, two equal flagella about the same length as the cell, single parietal chloroplast with a lateral pyrenoid, an anterior ellipsoidal stigma, two anterior contractile vacuoles, a single posterior nucleus (Fig. 48.19). Vegetative division forming diads and mostly tetrads of daughter cells which remain closely associated with parental cell wall (Fig. 48.14).

Distribution: Recorded from Marie Byrd Land, Scott Base, Cape Crozier and Granite Harbour.

Fig. 48. ***Tetracystis.***

1-9. Morphotype 1. 1-2, cells showing lobed chloroplast with a pyrenoid. 3, three tetrads held together by thin, firm mucilage. 4, chloroplast stripe-like in surface view. 5, aplanosporangium with four aplanospores. 6, diad stage. 7, pyrenoids with thick, entire, perforated and lobed starch sheaths. 8, ellipsoidal aplanospore. 9, zoospores with flat or a hump-like papilla, posterior nucleus, median to anterior stigma and two equal flagella.

10-21. Morphotype 2. 10-12. spherical cells showing lobed to cup-shaped chloroplast with fissured margin. 13, chloroplast stripe-like in surface view. 14, diad stage. 15, 16, tetrad complexes. 17, aplanosporangium containing six aplanospores. 18, ellipsoidal aplanospore. 19, zoospores with flat or two hump-like papillae, posterior nucleus, anterior stigma and two equal flagella. 20, pyrenoids with entire, perforated and lobed starch sheaths. 21, tetrad with mucilage sheath.

Scale bar is 10 μm.

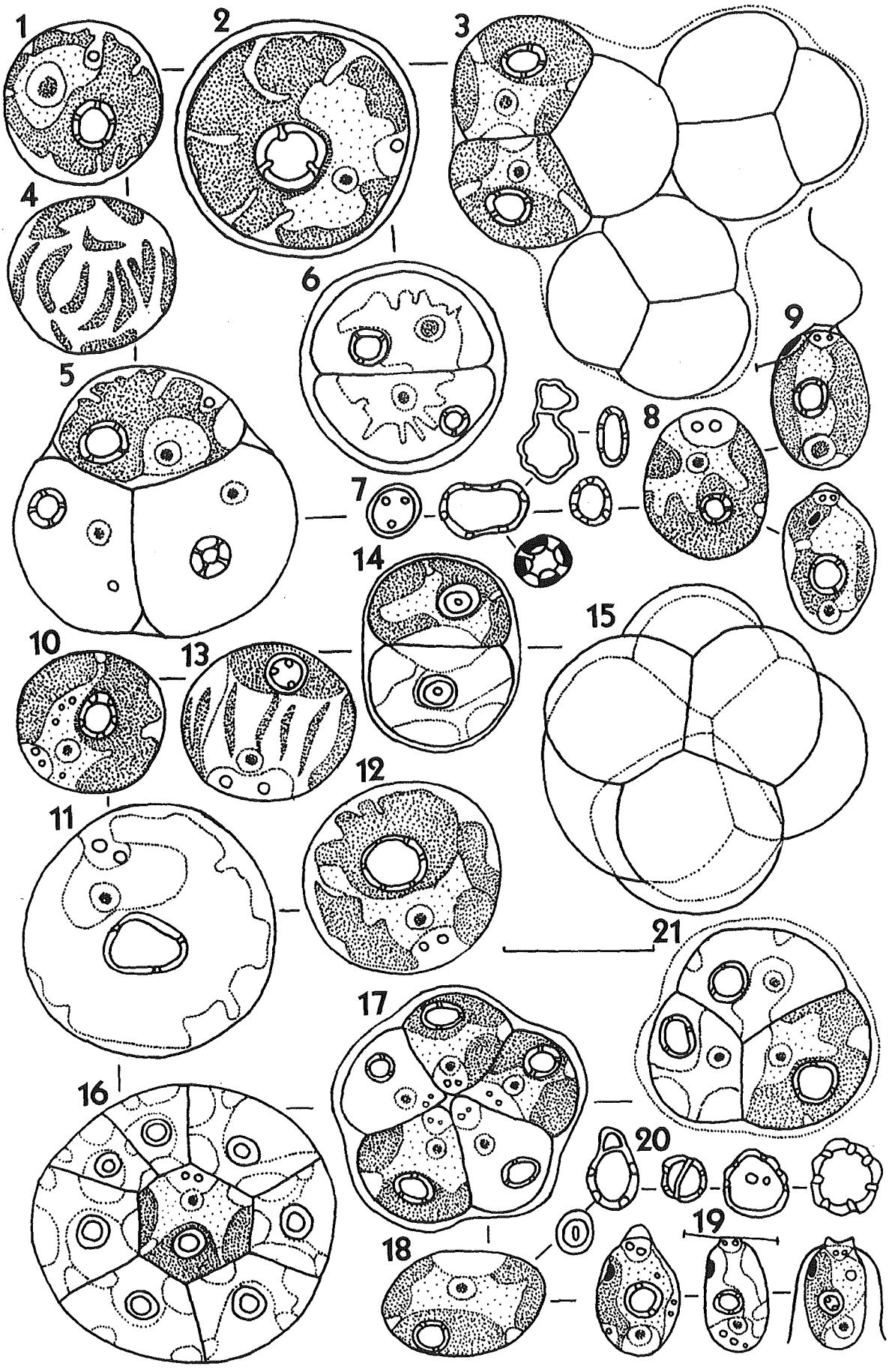
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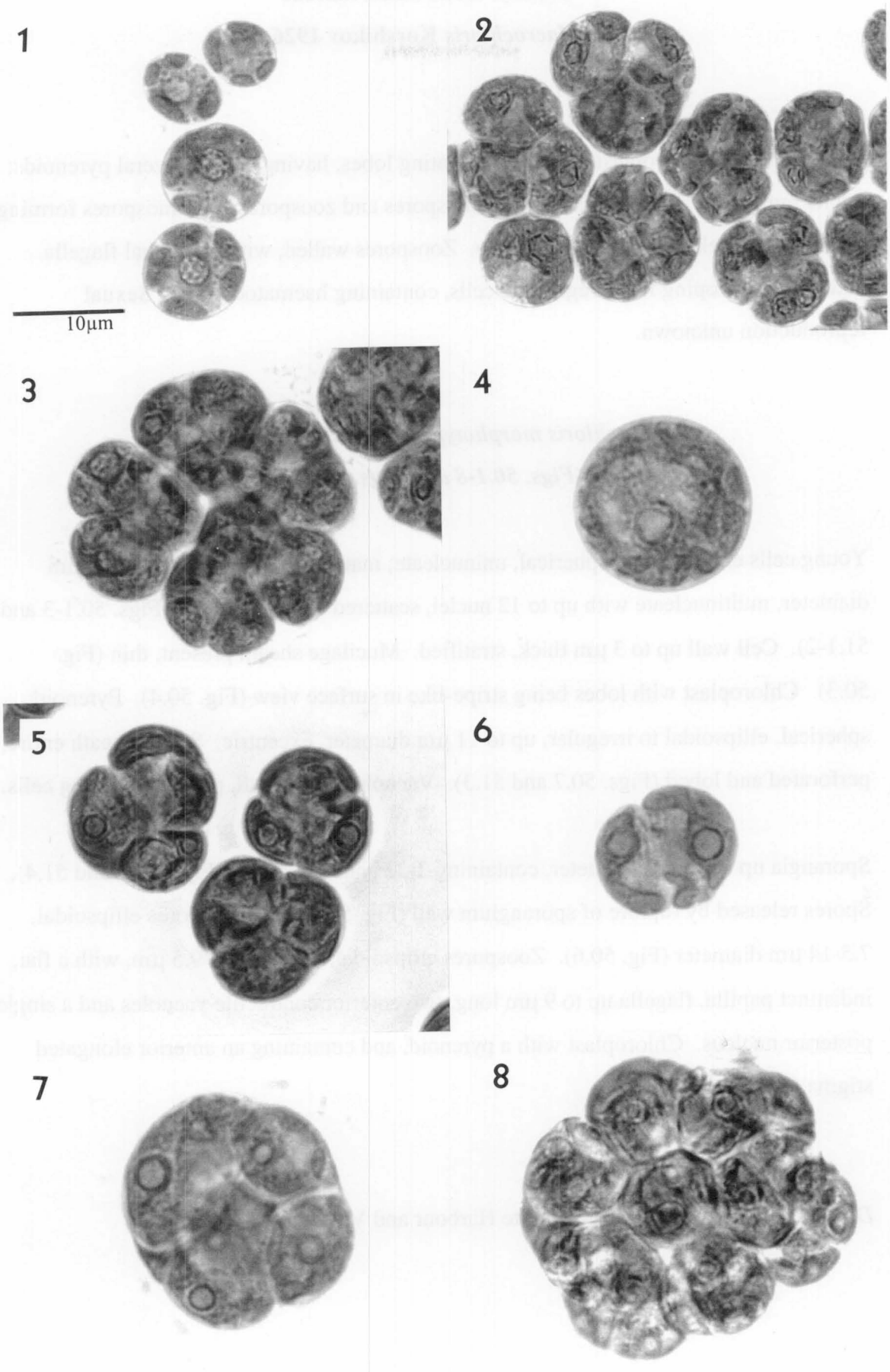
Fig. 49. *Tetracystis*, LM.

1-3. Morphotype 1. 1, cells with lobed chloroplast. 2, several loosely clustered tetrads. 3, four tetrads loosely held together by mucilage sheath.

4-8. Morphotype 2. 4, adult cell. 5, loosely associated tetrads. 6, aplanosporangium with two aplanospores. 7, aplanosporangium containing six aplanospores. 8, cell complex formed by vegetative division.

Scale in 1 also applies to 2-8.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Family: Actinochloridaceae

Macrochloris Korshikov 1926

Cells spherical, chloroplast axial with radiating lobes, having one or several pyrenoids; nuclei numerous. Reproduction by aplanospores and zoospores. Aplanospores forming a complex of cells which later separate. Zoospores walled, with two equal flagella. Akinetes developing from vegetative cells, containing haematochrome. Sexual reproduction unknown.

Macrochloris morphotype 1(M. cohaerens forma)
(Figs. 50.1-8 and Figs. 51.1-5)

Young cells ellipsoidal to spherical, uninucleate; mature cell spherical, 10-43.5 μm diameter, multinucleate with up to 12 nuclei, scattered throughout cell (Figs. 50.1-3 and 51.1-2). Cell wall up to 3 μm thick, stratified. Mucilage sheath present, thin (Fig. 50.3). Chloroplast with lobes being stripe-like in surface view (Fig. 50.4). Pyrenoid spherical, ellipsoidal to irregular, up to 11 μm diameter, excentric. Starch sheath entire, perforated and lobed (Figs. 50.7 and 51.3). Vacuoles few, small, present in young cells.

Sporangia up to 35 μm diameter, containing 2-32 and >32 spores (Figs. 50.8 and 51.4). Spores released by rupture of sporangium wall (Fig. 51.5). Aplanospores ellipsoidal, 7.5-14 μm diameter (Fig. 50.6). Zoospores ellipsoidal, 8-15 by 5.5-9.5 μm, with a flat, indistinct papilla, flagella up to 9 μm long, two anterior contractile vacuoles and a single posterior nucleus. Chloroplast with a pyrenoid, and containing an anterior elongated stigma (Fig. 50.5).

Distribution: Recorded from Granite Harbour and Victoria Valley.

Class: Chlorophyceae

Order: Chlorellales

Family: Myrmeciaceae

***Myrmecia* Printz 1921**

Cells solitary, free-living, spherical and ellipsoidal. Cell wall thin to thick, with or without polar thickening. Chloroplast parietal, without pyrenoid. Reproduction by naked, equally biflagellate zoospores, aplanospores (2-128) and autospores(4-8).

Myrmecia morphotype 1 (M. macronucleata (Deason) Andreeva)
(Figs. 52.40-46 and 51.6-8)

Cells 8-20 μm diameter (Figs. 52.40, 42 and 51.6). Nucleus single, large. Cell wall thin, occasionally up to 2 μm thick, stratified. Chloroplast mostly bi-lobed, occasionally tri-lobed (Figs. 52.40, 42 and 51.7).

Sporangia up to 23.5 μm diameter, containing 4, 8, 16 and >16 spores (Figs. 52.43, 46). Spores released by rupture of sporangium wall, tending to remain in temporary clusters after release (Fig. 52.41; Fig. 51.8). Aplanospores frequent, mostly spherical, occasionally ellipsoidal, 2.5-10 μm diameter (Fig. 52.45). Zoospores rarely formed, ellipsoidal to spindle-shaped with narrow anterior, 8-15 by 5.5-9.5 μm, becoming spherical after quiescence, flagella exceeding the cell length, two anterior contractile vacuoles, and a single median nucleus. Chloroplast containing an indistinct median stigma (Fig. 52.44).

Distribution: Recorded from Signy Island.

Family: Chlorellaceae

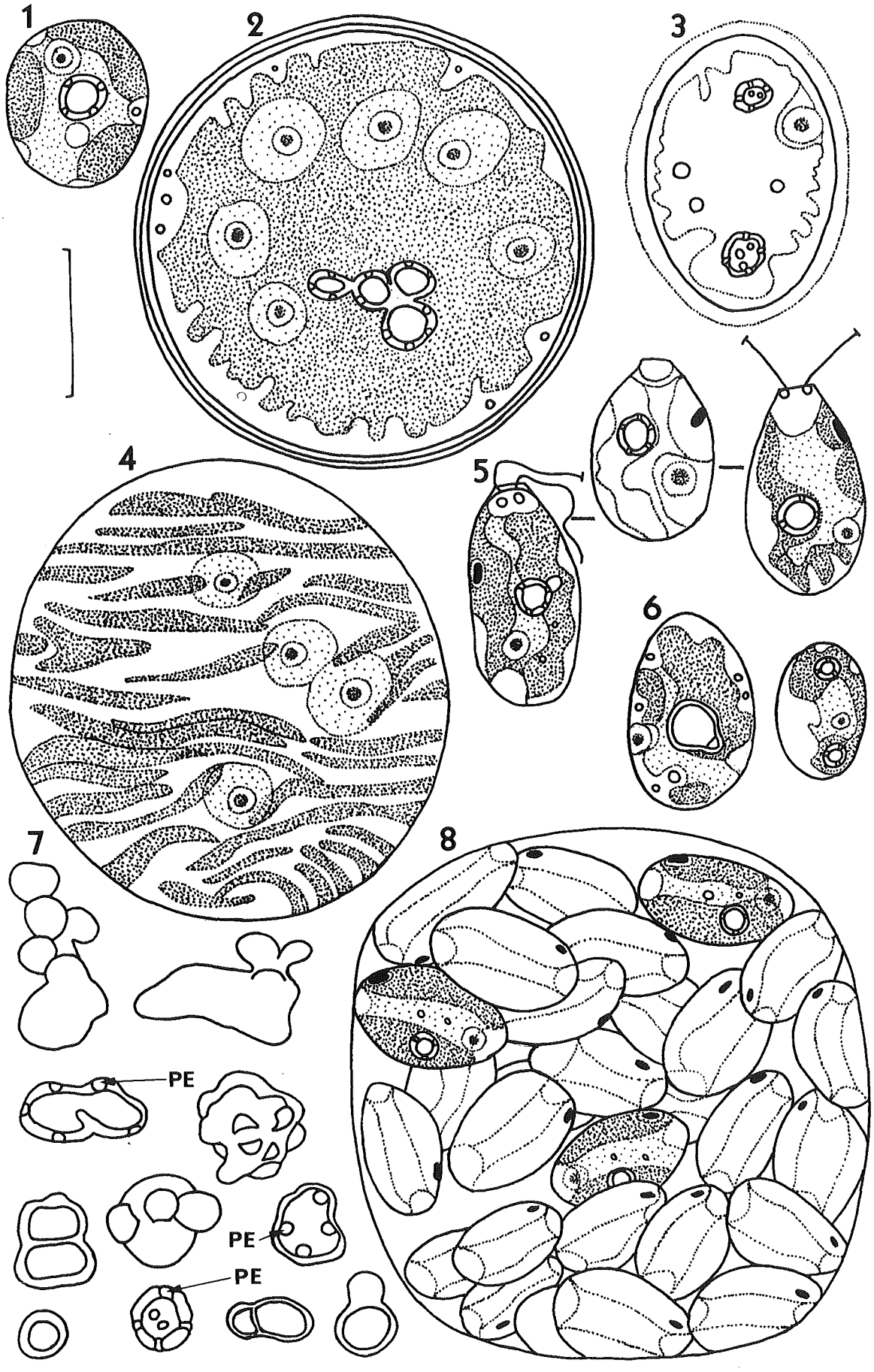
***Chlorella* Beijerinck 1890**

Cells solitary, or in small irregular groups, spherical to ellipsoidal, with distinct cell wall without mucilage. Chloroplast parietal, cup-shaped or plate of variable extent,

Fig. 50. *Macrochloris* Morphotype 1.

1, young, uninucleate cell. 2, adult cell with lobed chloroplast and scattered nuclei. 3, cell with two pyrenoids and mucilage sheath. 4, chloroplast stripe-like in surface view. 5, zoospores with indistinct flat papilla, posterior nucleus, anterior stigma and two equal flagella. 6, ellipsoidal aplanospores. 7, variation in shape and perforation (PE) of the starch sheath surrounding the pyrenoid. 8, zoosporangium containing numerous zoospores each with a distinct anterior stigma in the chloroplast.

Scale bar is 10 μm.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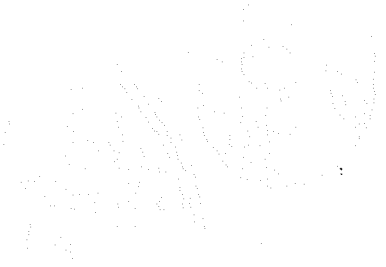
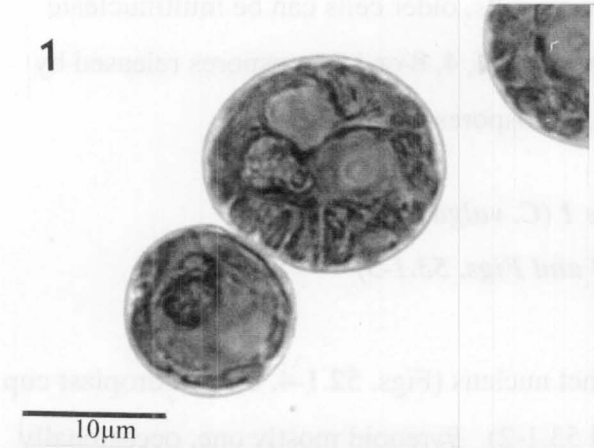


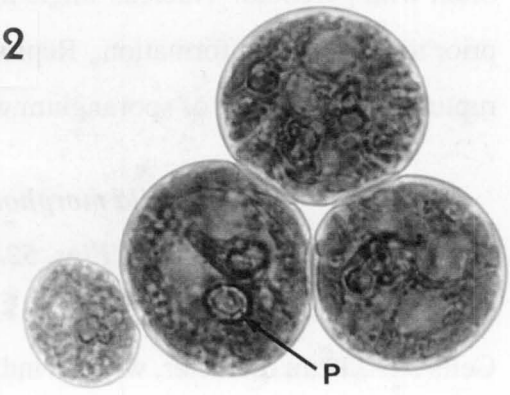
Fig. 51. ***Macrochloris*** **and** ***Myrmecia,*** **LM.**

1-5. *Macrochloris* morphotype 1. 1. vegetative cells with lobed chloroplast. 2, group of vegetative cells, one cell showing pyrenoid (P) with perforated starch sheath. 3, single pyrenoid with lobed starch sheath (SS) retained within the wall of a dead cell. 4, aplanosporangium containing numerous aplanospores. 5, ruptured aplanosporangium with single aplanospore.

6-8. *Myrmecia* morphotype 1. 6, vegetative cell with large nucleus. 7, vegetative cell with lobed chloroplast and large nucleus (N). 8, temporary cluster of aplanospores soon after release from aplanosporangium.

Scale in 1 also applies to 2-8.





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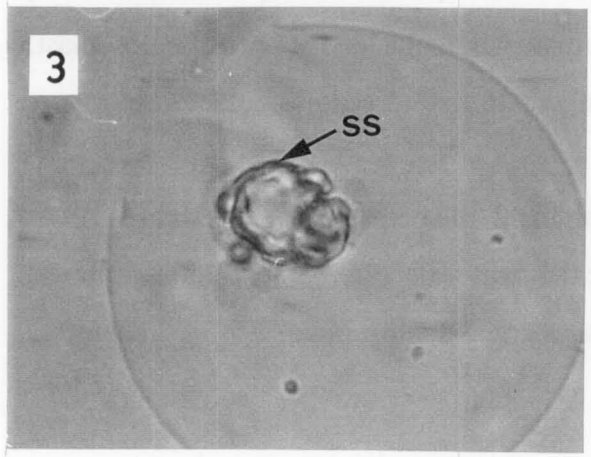

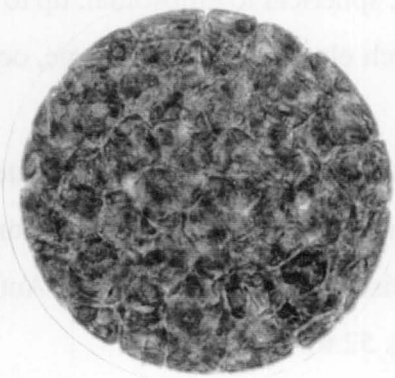

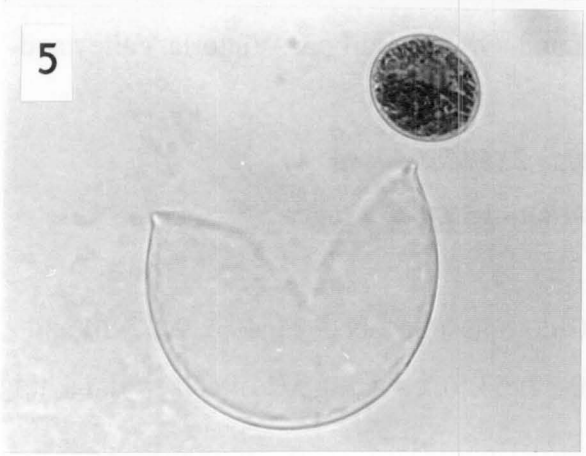

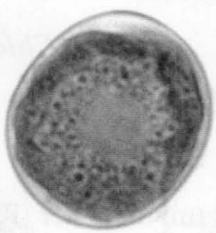

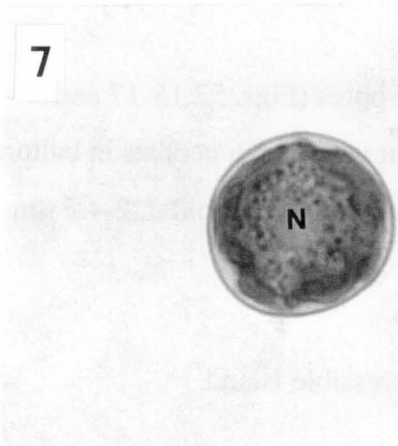

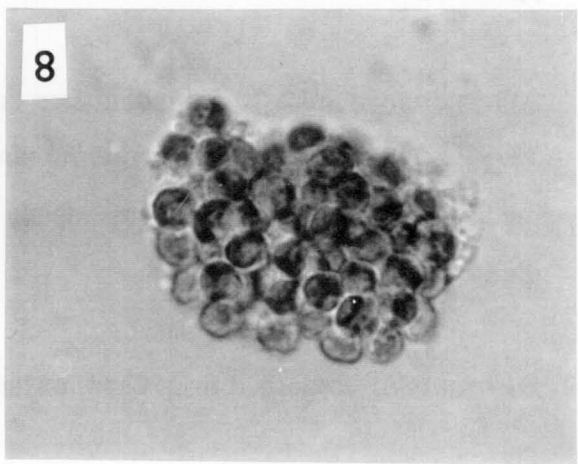

often with pyrenoid. Nucleus single in young cells, older cells can be multinucleate prior to sporangium formation. Reproduction by 2, 4, 8 or 16 autospores released by rupture or dissolution of sporangium wall. Zoospores lacking.

Chlorella morphotype 1 (C. vulgaris Beijerinck)
(Figs. 52.1-8 and Figs. 53.1-3)

Cells 3.5-11 μm diameter, with an indistinct nucleus (Figs. 52.1-4, 8). Chloroplast cup, girdle to band-shaped (Figs. 52.1-4, 8 and 53.1-2). Pyrenoid mostly one, occasionally two, spherical to ellipsoidal, up to 2.5 μm diameter, embedded at base of chloroplast. Starch plates mostly two, large, occasionally up to five. Vacuoles numerous.

Autosporangia up to 9 μm diameter, containing 2-16 spores (Figs. 52.5 and 53.3). Spores released by irregular rupture of sporangium wall (Fig. 52.7). Sporangium wall persisting in culture medium. Autospores spherical to ellipsoidal, 2.5-5 μm diameter (Fig. 52.6).

Distribution: Recorded from Marie Byrd Land, Granite Harbour, Victoria Valley and Edmonson Point.

Chlorella morphotype 2 (Chlorella sp. A)
(Figs. 52.9-18 and 53.4-6)

Cells 3-8.5 μm diameter (Figs. 52.9-14). Chloroplast bi-lobed (Figs. 52.9, 13-14 and 53.4), cup-shaped in side view (Figs. 52.10-12; 53.5), covering 3/4 of cell wall, lacking pyrenoid.

Autosporangia up to 10.5 μm diameter, containing 2-16 spores (Figs. 52.15-17 and 53.6). Spores released by irregular rupture of sporangium wall which persists in culture medium (Fig. 52.18). Autospores mostly spherical, occasionally ellipsoidal, 2-4.5 μm diameter.

Distribution: Recorded from Granite Harbour and Inexpressible Island.

Chlorella morphotype 3 (C. cf. luteoviridis)
(Figs. 52.19-23 and Figs. 53.7-9)

Cells 7.5-16 μm diameter (Figs. 52.19, 22). Chloroplast cup (Figs. 52.19; 53.8) to band-shaped (Figs. 52.22; 53.7) with lobed margin, covering 1/2 to 3/4 of cell wall. Pyrenoid one to four (Fig. 52.23), ellipsoidal to spherical, up to 3.5 μm diameter, embedded at base of chloroplast. Starch plates two to five. Vacuoles one to numerous.

Autosporangia up to 18 μm diameter, containing 2-16 spores (Figs. 52.20 and 53.9). Spores released by irregular rupture of sporangium wall (Fig. 52.21). Autospores spherical to ellipsoidal, 4-11.5 μm diameter, unequal sizes in each sporangium, with one spore larger than others (Fig. 53.9).

Distribution: Recorded from Victoria Valley.

Chlorella morphotype 4 (C. emersonii Shihira and Krauss)
(Figs. 52.24-31 and 53.10-12)

Cells 4-11.5 μm diameter (Figs. 53.10-11). Chloroplast parietal, cup-shaped (Fig. 52.25), perforated (Figs. 52.24, 31), lobed (Fig. 52.26), deep incisions between lobes in surface view (Fig. 52.27). Pyrenoid mostly one, occasionally two, spherical, up to 2 μm diameter, embedded at base of chloroplast. Starch plates two to four.

Autosporangia up to 9.5 μm diameter, containing 2-100 spores, 4 autospores frequent (Figs. 52.30 and 53.12). Spores released by irregular rupture of sporangium wall (Fig. 52.28). Autospores spherical to ellipsoidal, 3.5-7.5 μm diameter (Fig. 52.29).

Distribution: Recorded from Edmonson Point.

Fig. 52. *Chlorella* and *Myrmecia.*

1-8. *Chlorella* morphotype 1. 1-2, 8, spherical cells with girdle and band-shaped chloroplasts with pyrenoids surrounded by two to four starch plates. 3, cell with cup-shaped chloroplast. 4, adult cell with saucer-shaped chloroplast that is compressed by a large vacuole. 5, autosporangium enclosing eight autospores. 6, ellipsoidal autospore. 7, irregular rupture of sporangium wall.

9-18. *Chlorella* morphotype 2. 9, 13-14, cells showing bi-lobed chloroplasts which lack pyrenoids. 10-12, chloroplasts cup-shaped in side view. 15, autosporangium containing 16 autospores. 16, autosporangium with two autospores. 17, autosporangium with four autospores. 18, irregular rupture of autosporangium wall.

19-23. *Chlorella* morphotype 3. 19, 22-23, adult cells. 19, cell with cup-shaped chloroplast with pyrenoid surrounded by five starch plates. 22, cell with band-shaped chloroplast and a large vacuole. 23, cells with band-shaped chloroplast with lobed margin and with four pyrenoids. 20, autosporangium containing unequal-sized autospores, one spore larger than others. 21, irregular rupture of sporangium wall.

24-31. *Chlorella* morphotype 4. 24, 31, spherical to ellipsoidal cells with perforated chloroplasts and pyrenoids with starch plates. 25, cell with cup-shaped chloroplast and pyrenoid with two starch plates. 26, cells with lobed chloroplast. 27, chloroplast in surface view, deep incisions between chloroplast lobes. 28, autospores released by irregular rupture of autosporangium wall. 29, autosporangium wall adherent to autospore. 30, autosporangium containing four autospores.

32-39. *Chlorella* morphotype 5. 32, cell with band-shaped chloroplast with pyrenoid surrounded by starch grains. 33, 35, cells with band-shaped chloroplasts with lobed margins and one to two pyrenoids. 34, cell with cup-shaped chloroplast. 36, 37, autosporangium containing unequal-sized autospores. 38, ellipsoidal autospore with band-shaped chloroplast. 39, autospores released by irregular rupture of autosporangium wall.

40-46. *Myrmecia* morphotype 1. 40, vegetative cell with bi-lobed chloroplast and single, large nucleus. 41, spores remaining in a temporary cluster after release. 42, vegetative cell with tri-lobed chloroplast and a large nucleus. 43, aplanosporangium. 44, zoospores ellipsoidal to spindle-shaped with median stigma and with two long flagella. 45, aplanospores with and without stigma. 46, aplanosporangium containing numerous aplanospores.

Scale bar is 10 μm.

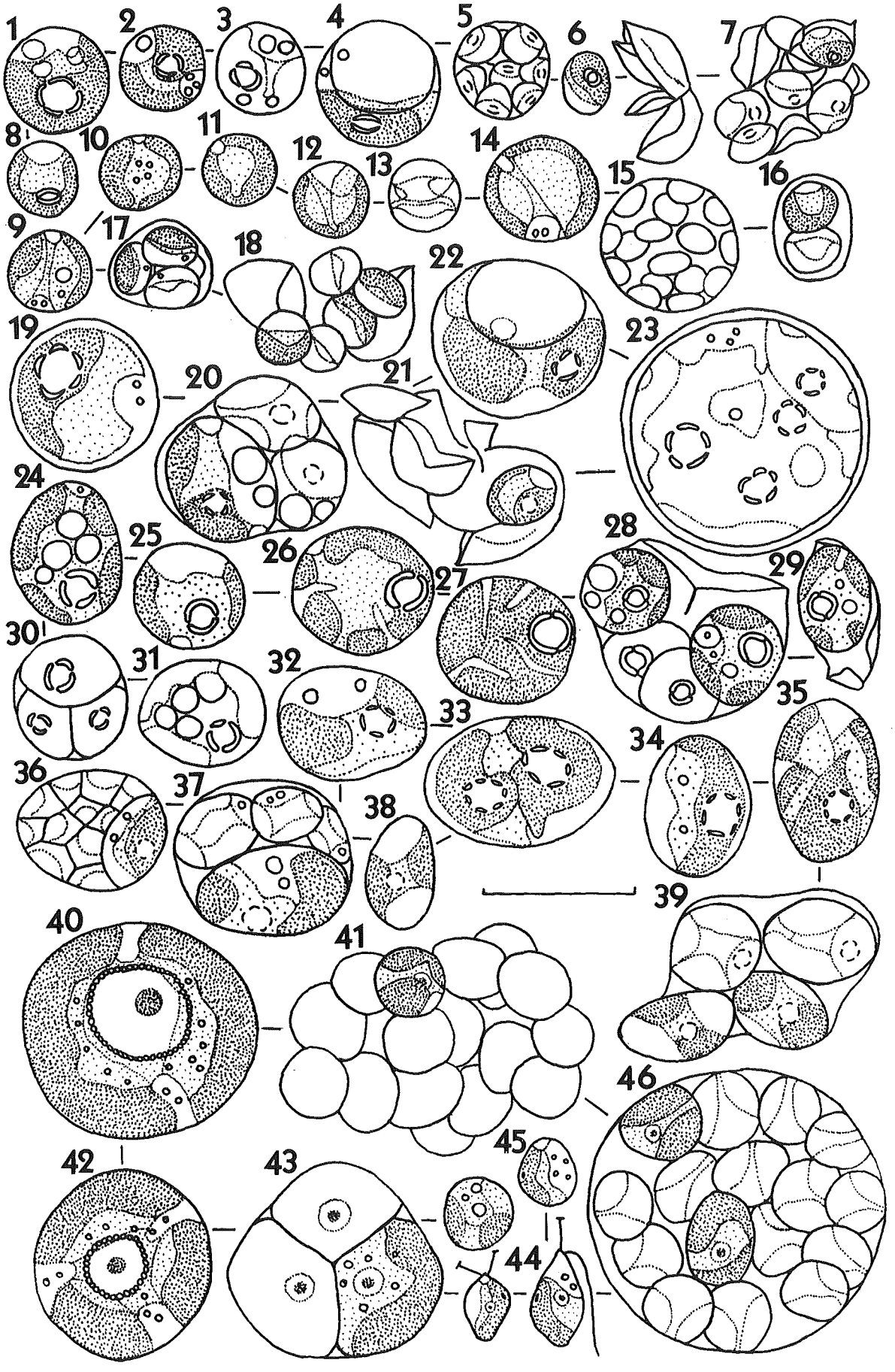
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Fig. 53. *Chlorella,* LM.

1-3. Morphotype 1. 1, cell with a large vacuole. 2, cell with cup-shaped chloroplast. 3, adult cells and an autosporangium (AU) containing four autospores.

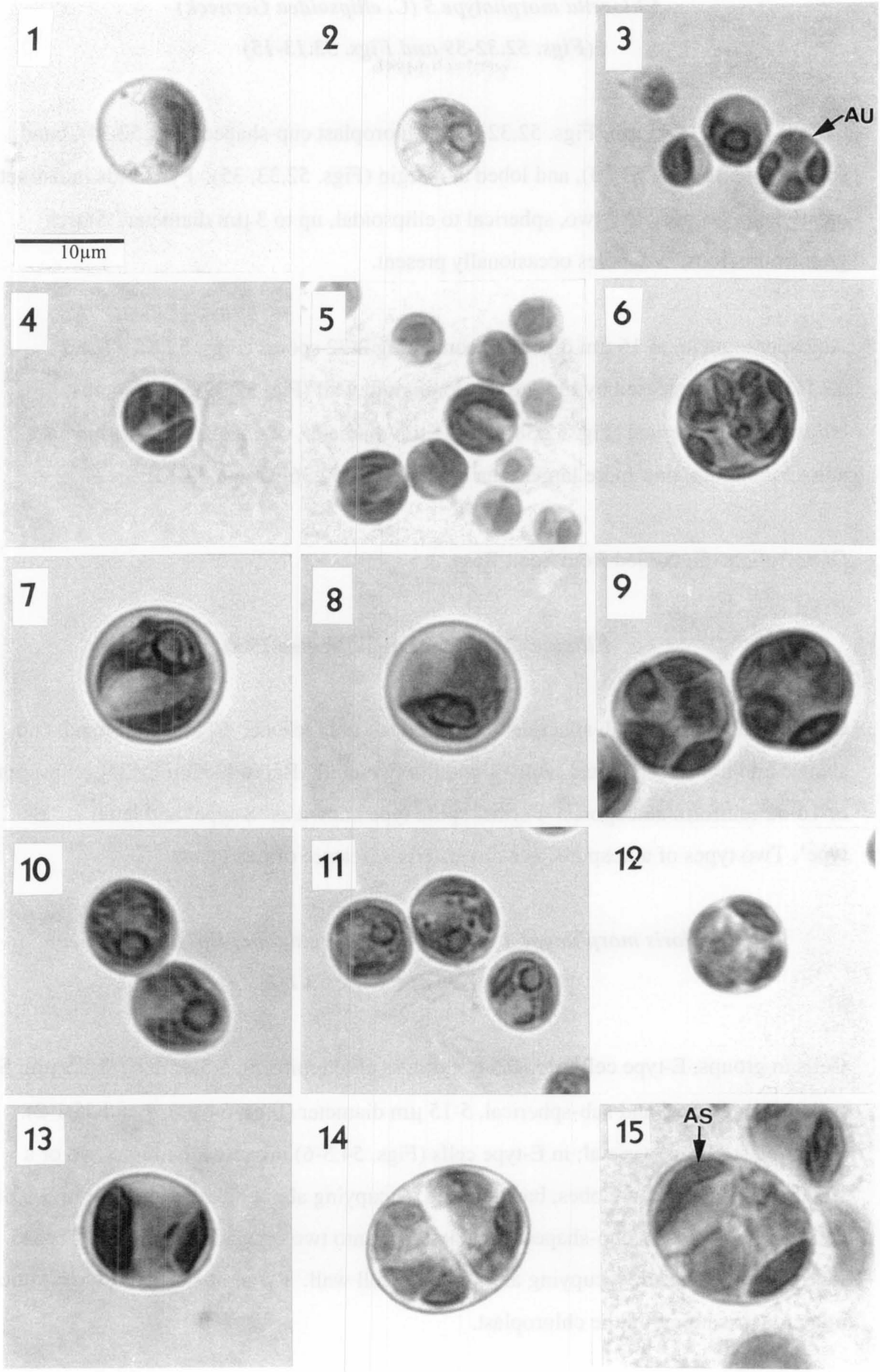
4-6. Morphotype 2. 4, cell with bi-lobed chloroplast. 5, chloroplasts cup-shaped in side view. 6, autosporangium containing four autospores.

7-9. Morphotype 3. 7, cell with band-shaped chloroplast and a pyrenoid. 8, cell with cup-shaped chloroplast and a pyrenoid. 9, two autosporangia.

10-12. Morphotype 4. 10-11, cells showing cup-shaped chloroplasts each with a single pyrenoid. 12, autosporangium.

13-15. Morphotype 5. 13, cell with band-shaped chloroplast; 14, autosporangium with two autospores. 15, autosporangium containing unequal sized autospores (AS), one larger than remainder.

Scale in 1 also applies to 2-15.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Chlorella morphotype 5 (C. ellipsoidea Gerneck)
(Figs. 52.32-39 and Figs. 53.13-15)

Cells 9-18 by 6-14.5 μm (Figs. 52.32-35). Chloroplast cup-shaped (Fig. 52.34), band-shaped (Figs. 52.32; 53.13), and lobed at margin (Figs. 52.33, 35). Pyrenoids indistinct, mostly one, occasionally two, spherical to ellipsoidal, up to 3 μm diameter. Starch grains numerous. Vacuoles occasionally present.

Autosporangia up to 16 μm diameter, containing 2-32 spores (Figs. 52.36-37 and 53.14). Spores released by rupture of sporangium wall (Fig. 52.39). Autospores ellipsoidal to spherical (Fig. 52.38), 3.5-11 μm diameter, of unequal size within an autosporangium, one spore larger than others (Figs. 52.36-37 and 53.15).

Distribution: Recorded from Scott Base.

Elliptochloris Tschermak-Woess 1980

Cells solitary, ellipsoidal, spherical to cylindrical, uninucleate. Chloroplast band, cup-shaped and hollow-spherical, with or without pyrenoid. Reproduction by 2-4 ellipsoidal or 16-32 reniform autospores. Former spore type known as 'S-type' and latter as 'E-type'. Two types of autospores is a characteristic feature of this genus.

Elliptochloris morphotype 1 (E. reniformis (Watanabe) Ettl and Gärtner)
(Figs. 54.1-15 and Figs. 55.1-2)

Cells in groups, E-type cells elongated, cylindrical to reniform, 5.5-12.5 by 2.5-8 μm, S-type cells spherical and sub-spherical, 5-15 μm diameter (Figs. 54.1-6, 9 and 55.1-2). Chloroplast single, parietal; in E-type cells (Figs. 54.3-6) broadly lobed into two or a small numbers of broad lobes, band-shaped, occupying about half of cell wall; in S-type cells (Figs. 54.1-2, 9) cup-shaped, broadly lobed into two or a small numbers of broad lobes, mantle-shaped, occupying almost entire cell wall. Pyrenoid absent. Starch grains numerous, scattered in the chloroplast.

Autosporangia S-type 6.5-11 μm diameter and E-type 9-17.5 by 6-10.5 μm. S-type sporangia (Figs. 54.10, 11) contain mostly 2-4 spores, occasionally up to 16 and E-type sporangia (Figs. 54.7, 8, 14) contain 2-16 spores, occasionally >16. Spores released by rupture of sporangium wall (Fig. 54.15). S-type autospores spherical, 3-7.5 μm diameter (Fig. 54.13) and E-type autospores ellipsoidal to reniform, 3.5-8 by 2-3.5 μm (Figs. 54.12 and 55.1).

Distribution: Recorded from Signy Island, Marie Byrd Land and Granite Harbour.

Elliptochloris morphotype 2 (E. reisiglii nov. comb.)
(Figs. 54.16-28 and 55.3-4)

Cells in groups, E-type cells elongated, pyriform to reniform, 5-9 by 2.5-6 μm, S-type cells spherical or sub-spherical, 4-7.5 μm diameter (Figs. 54.16-20, 26 and 55.3-4). Chloroplast lobed, band to cup-shaped in both cell types, occupying about half of cell wall (Figs. 54.16-20, 26). Pyrenoids ellipsoidal, mostly one, occasionally up to four. Starch grains small, numerous.

Autosporangia S-type up to 8 μm diameter (Fig. 55.4) and E-type up to 9 by 6 μm. S-type sporangia (Figs. 54.23, 25) contain 2-4 spores and E-type sporangia (Figs. 54.21, 22) contain 2-8 spores. Spores released by irregular rupture of sporangium wall which remains adherent to spores. S-type autospores (Fig. 54.27) spherical, 2.5-4 μm diameter, E-type autospores ellipsoidal to reniform, 3.5-6 by 1.5-3.5 μm (Fig. 54.28).

Distribution: Recorded from Granite Harbour.

Family: Neochloridaceae
***Bracteacoccus* Tereg 1923**

Cells solitary, free-living, spherical to ellipsoidal, multinucleate. Chloroplasts numerous in adult cells, few in young cells, parietal, plate-like, lacking pyrenoid.

Reproduction by aplanospores or zoospores, the latter being naked and with two slightly unequl flagella.

Bracteacoccus morphotype 1 (B. aerius Bischoff and Bold)
(Figs. 54.29-36 and Figs. 55.5-8)

Cell 7.5 to 23 μm diameter (Figs. 54.29-31 and 55.5-6). Cell wall smooth, mostly thin, occasionally up to 2 μm thick. Chloroplast numerous (>20).

Sporangia up to 15 μm diameter, containing 4-16 or >16 spores (Figs. 54.34, 36; Figs. 55.7-8). Spores released by rupture of sporangium wall (Figs. 54.35 and 55.8). Aplanospores spherical, 3-6 μm diameter, with one to two chloroplasts (Figs. 54.33 and 55.8), tending to remain in temporary, loose aggregates after release. Zoospores ovoidal to ellipsoidal, with posterior pointed, 3.5-5.5 by 2.5 -3.5 μm, with two apical contractile vacuoles, an anterior nucleus, longer flagellum up to 7μm and smaller up to 6 μm long, with one or two chloroplasts containing a posterior stigma (Fig. 54.32).

Distribution: Recorded from Granite Harbour.

Order: Gloeotilales
Family: Gloeotilaceae
***Binuclearia* Wittrock 1887**

Filaments unbranched, without a broad gelatinous envelope. Cells cylindrical, uninucleate, with thick lamellate walls, marked by thickened and prominently stratified transverse septa which are like H-shaped. Chloroplast parietal, girdle-shaped, covering usually 3/4 circumference of the cell, but often only a part of the length. Pyrenoid present. Reproduction by zoospores aplanospores, fragmentation and also by akinetes.

Binuclearia morphotype 1 (B. tectorum forma)
(Figs. 54.37-44; 56.1-7 and 57.1-6)

Filaments enclosed in a thin to up to 1μm thick mucilage sheath (Figs. 54.41 and 57.1),

rounded apical cell covered with bell-shaped cap of mucilage (Fig. 54.38). Cells cylindrical to ellipsoidal, occasionally median region slightly constricted, 7.5-28.5 by 2.5-7.5 μm (Fig. 54.37). Cell wall smooth, stratified, especially distinct in old or dead cells, remains of cell walls of mother cells form H-pieces (Fig. 54.39). Chloroplast girdle or band-shaped, covering 2/3 of the cell circumference. Pyrenoid spherical to ellipsoidal (Fig. 57.1), situated in thick, median region of chloroplast, occasionally two. Starch grains two to six. A large vacuole at each pole of the cell (Fig. 54.37).

Sporangia up to 28.5 long by 7.5 μm wide, containing 1-4 spores (Fig. 54.40). Spores released through a round opening or transverse rupture of sporangium wall (Fig. 54.42), and adhering to a mucilagenous disc (Figs. 56.6-7). Aplanospores spherical, 3.5-7 μm diameter (Figs. 54.44 and 57.2). Zoospores spherical to pyriform, 3.5-7 by 3.5-6 μm, equally quadriflagellate, flagella up to 8 μm long, nucleus anterior, no contractile vacuole, chloroplast cup-shaped with pyrenoid, and a median to posterior, ellipsoidal, distinct stigma (Fig. 54.43). Akinetes mostly spherical, occasionally ellipsoidal, up to 26 μm diameter, with stratified, thick cell wall up to 1μm diameter, with band-shaped chloroplast, full of granules and with a large vacuole (Figs. 56.1-2 and 57.2-3). Germination of akinetes observed in old cultures (48 day) followed by cell division to produce a filament (Figs. 56.3-5 and 57.4-6).

Distribution: Recorded from Scott Base.

Order: Chaetophorales

Family: Chaetophoraceae

Sub-family: Ulvelloideae

***Protoderma* Kützing 1843**

Thallus consists of a small, central group of polygonal, pseudoparenchymatous, irregularly arranged cells from which short, branching filaments extend in all directions.

Fig. 54. ***Elliptochloris, Bracteacoccus*** **and** ***Binuclearia.***

1-15. *Elliptochloris* morphotype 1. 1-2, 9, spherical and subspherical S-type adult cells with cup-shaped and lobed chloroplasts. 3-6, cylindrical to reniform E-type cells with lobed and band-shaped chloroplast. 7, 8, E-type sporangia containing S-type autospores. 10, S-type sporangium containing S-type spores. 11, S-type sporangium containing E-type spores. 12, ellipsoidal to reniform E-type autospores. 15, ruptured sporangium.

16-28. *Elliptochloris* morphotype 2. 16-17, spherical and subspherical S-type adult cells with cup-shaped and lobed chloroplasts with pyrenoid. 18-20, cylindrical, reniform to pyriform E-type cells with cup-shaped and lobed chloroplasts with two pyrenoids. 21, E-type sporangia containing E-type autospores. 22, E-type sporangium containing S-type spores. 23, S-type sporangium containing four S-type spores. 24, spores released by rupture of sporangium wall. 25, S-type sporangium containing two E-type spores. 26, E-type young cell. 27, spherical S-type autospore. 28, ellipsoidal to reniform E-type autospores.

29-36. *Bracteacoccus* morphotype 1. 29-30, cells with plate-like to polygonal chloroplasts. 31, multinucleate adult cell. 32, zoospores ovoidal and ellipsoidal with pointed posterior, anterior nucleus, posterior stigma and two, slightly unequal flagella. 33, spherical aplanospore. 34, aplanosporangium containing numerous aplanospores. 35, spores released by rupture of sporangium wall. 36, aplanosporangium containing eight aplanospores.

37-44. *Binuclearia* morphotype 1. 37, cylindrical cell containing girdle-shaped chloroplast; pyrenoid with starch grains; polar vacuoles each with one refractive globule; single nucleus. 38, apical cell with cap of mucilage sheath. 39, empty cell with H-pieces clearly seen. 40, formation of spores each with a distinct stigma. 41, filament with thin, mucilage sheath. 42, release of aplanospores by transverse rupture of sporangium wall. 43, zoospores spherical and ellipsoidal with pointed anterior end, broad posterior end, anterior nucleus, median to posterior stigma and four equal flagella. 44, spherical aplanospore with stigma.

Scale bar is 10 μm.

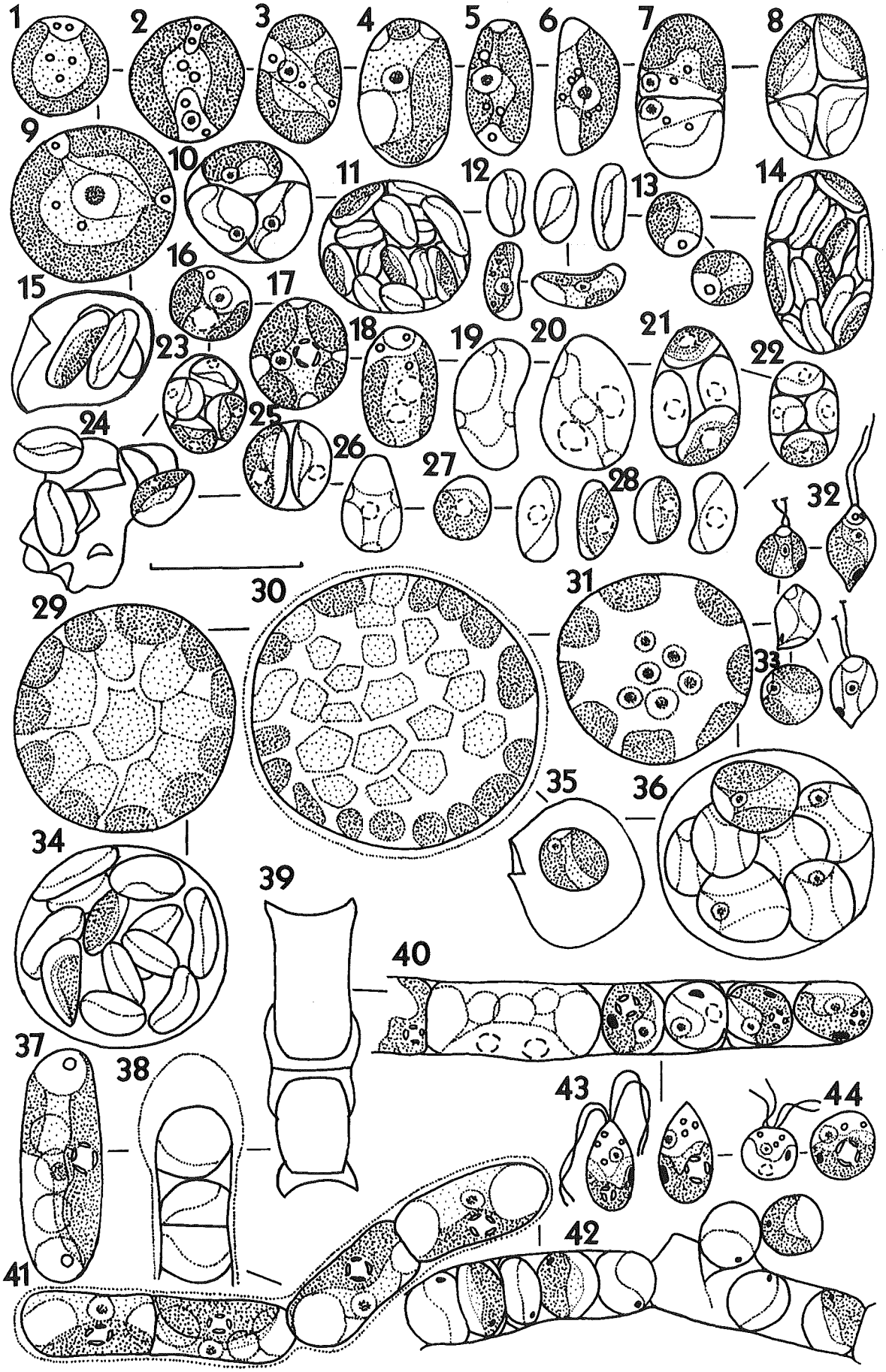
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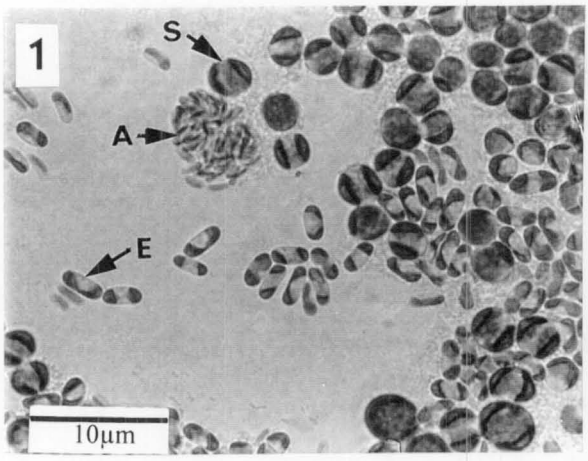
Fig. 55. *Elliptochloris* and *Bracteacoccus*, LM.

1-2. *Elliptochloris* morphotype 1. 1, spherical S-type (S) adult cells, ellipsoidal E-type (E) cells and cluster of E-type autospores (A). 2, spherical and reniform cells.

3-4. *Elliptochloris* morphotype 2. 3, ellipsoidal E-type cells with parietal chloroplast and pyrenoid (P). 4, spherical autosporangia (SA) containing two to four ellipsoidal autospores, ellipsoidal autosporangium (EA) with two ellipsoidal autospores and with spherical and ellipsoidal cells.

5-8. *Bracteacoccus* morphotype 1. 5, group of young cells; 6, two adult cells with polygonal chloroplasts. 7, aplanosporangia containing numerous aplanospores. 8, aplanosporangia with spherical aplanospores and spores released by rupture of sporangium wall.

Scale in 1 also applies to 2-8.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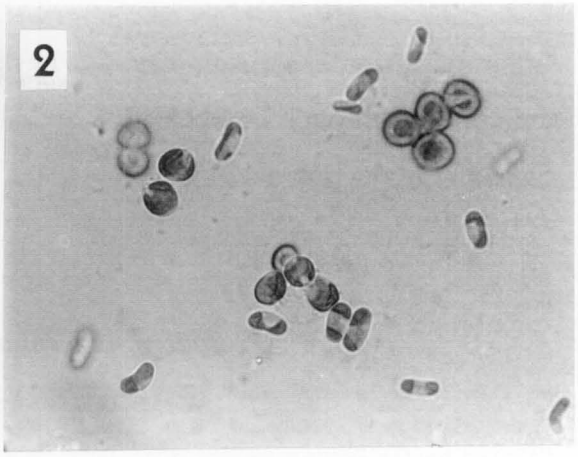
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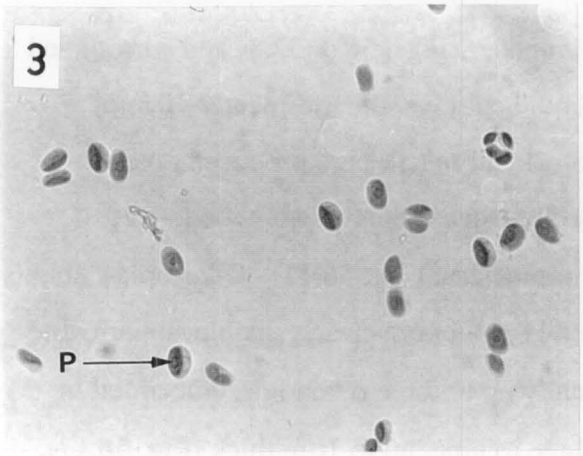
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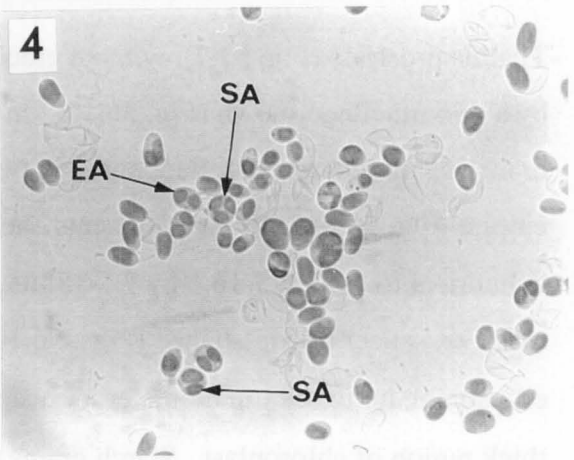
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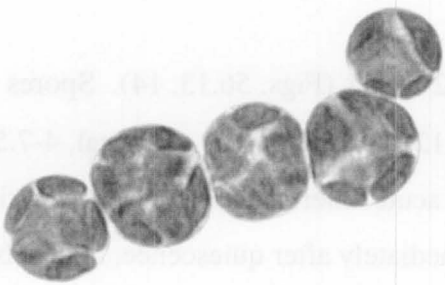
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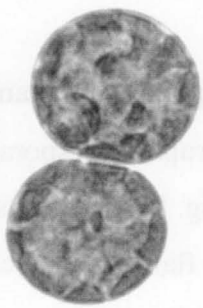
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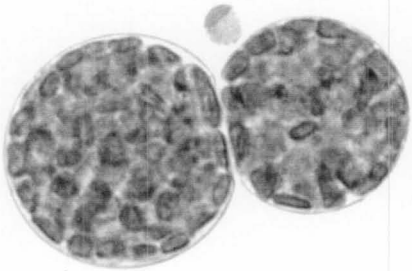
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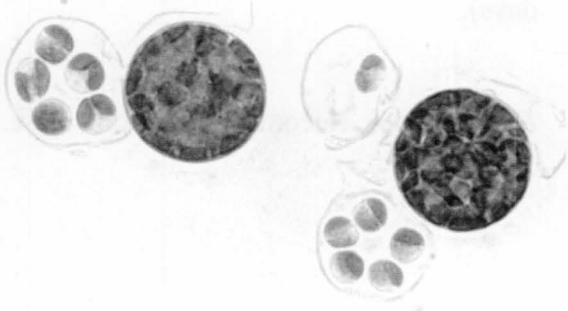
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Chloroplast single, parietal, plate-like or flattened, with a single pyrenoid. Asexual reproduction through fragmentation of thallus, biflagellate zoospores and aplanospores. Sexual reproduction not observed.

Protoderma morphotype 1 (cf. Protoderma)

(Figs. 56.8-17 and 57.1-2)

Thallus prostrate (Fig. 52.7), without erect branches (Figs. 56.9, 57.8) and surrounded by a thin mucilage sheath (Fig. 56.10). In liquid cultures, the rudimentary lateral branches are two to six-celled, but only two-celled branches are produced on agar culture (Fig. 56.8). Primary filaments one-celled, occasionally two-celled. Cell cylindrical to oval, 8.5-16.5 by 7.5-15 μm, uninucleate (Fig. 56.11). Chloroplast single, massive, saucer to cup-shaped (Figs. 56.10, 11). Pyrenoid clearly visible, spherical to ellipsoidal, up to 4.8 μm diameter, occasionally up to three pyrenoids, embedded in thick region of chloroplast. Starch grains three to nine, up to 1μm thick (Fig. 56.17). Vacuoles occasionally present.

Sporangia up to 20 μm diameter, containing 2-12 spores (Figs. 56.13, 14). Spores released by rupture of sporangium wall (Fig. 56.12). Aplanospores spherical, 4-7.5 μm diameter (Fig. 56.16). Zoospores pyriform with acute anterior and rounded posterior, no dorsiventral flattening, no changes in shape immediately after quiescence, 5-10.5 by 2.5-4.5 μm, flagella about same length as cell, single anterior contractile vacuole, and a single median to posterior nucleus. Chloroplast band-shaped with a pyrenoid, and containing an anterior elongated stigma. Zoospores found only in old culture (up to 40 days).

Distribution: Recorded from Scott Base.

Class: Ulvophyceae

Order: Pleurastrales

Family: Pleurastraceae

***Trebouxia* Puymaly 1924**

Cells solitary, free-living or usually as phycobiont in lichens. Chloroplast large, axial, stellate, containing central or slightly lateral pyrenoid. Nucleus in lateral position. Reproduction by autospores or naked, equally biflagellate zoospores.

Trebouxia morphotype 1 (T. corticola forma)

(Figs. 58.1-9 and 59.1-4)

Cells single or in loose groups, spherical (Figs. 59.1), 10.5-21 μm diameter, with a distinct nucleus (Fig. 59.3). Chloroplast lobed (Figs. 58.1, 4 and 59.2), stripe-like in surface view (Fig. 58.2). Pyrenoids indistinct, irregular (Fig. 58.7), usually one or two, occasionally up to seven, embedded at centre of chloroplast. Starch sheath entire.

Sporangia up to 24 μm diameter, containing 2-16 spores (Figs. 58.3, 5-6; Fig. 59.4). Spores released by irregular rupture of sporangium wall. Autospores frequent, spherical, 6.5-12.5 μm diameter (Fig. 58.9). Zoospores spherical to pyriform, 4-7.5 by 3.5-6 μm, flagella about same length as cell, and a single median to anterior nucleus. Chloroplast with a pyrenoid, and containing an anterior minute stigma (Fig. 58.8).

Distribution: Recorded from Victoria Valley.

Trebouxia morphotype 2 (T. crenulata Archibald)

(Figs. 58.10-19 and 59.5-8)

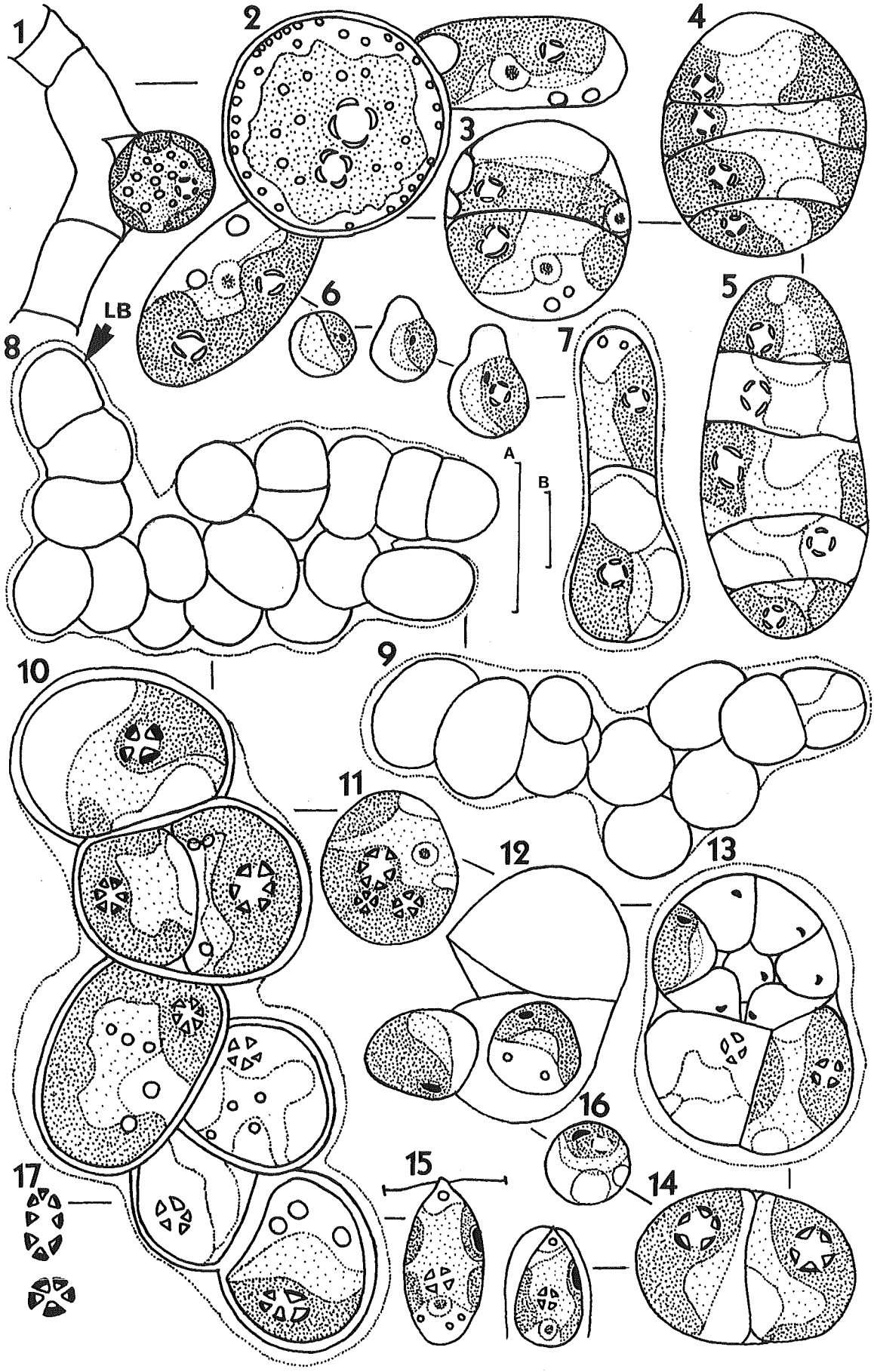
Cells single or in groups of two or four, spherical (Figs. 58.10-11), 6-22.5 μm diameter, with a distinct nucleus, multinucleate before cytokinesis, with or without contractile vacuoles. Chloroplast crenulate, lobed (Figs. 58.12 and 59.5). Pyrenoid indistinct,

Fig. 56. *Binuclearia* and *Protoderma.*

1-7. *Binuclearia* morphotype 1. 1, release of an akinete through ruptured sporangium wall. 2, spherical akinete with thick cell wall and granules. 3-5, germination of akinete to form short filament. 6-7, germinating stages of spores.

8-17. *Protoderma* morphotype 1. 8, young thallus with lateral branch (LB) two cells long, form on agarised culture. 9, young thallus without erect branching. 10, portion of mature thallus with mucilage sheath and saucer to cup-shaped chloroplast. 11, adult cell with three pyrenoids; 12, spores released by rupture of sporangium wall. 13, group of cells including a developed sporangium. 14, aplanosporangium containing two aplanospores. 15, zoospores with acute anterior and rounded posterior end, median to posterior nucleus, anterior stigma and two equal flagella. 16, spherical aplanospore with stigma. 17, spherical to ellipsoidal pyrenoids each surrounded by several, thick starch grains.

Scales are 10 μm, B applies to 8-9 and A to 1-7 and 10-17.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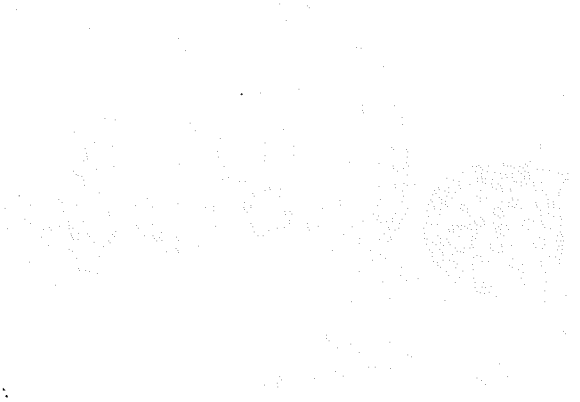
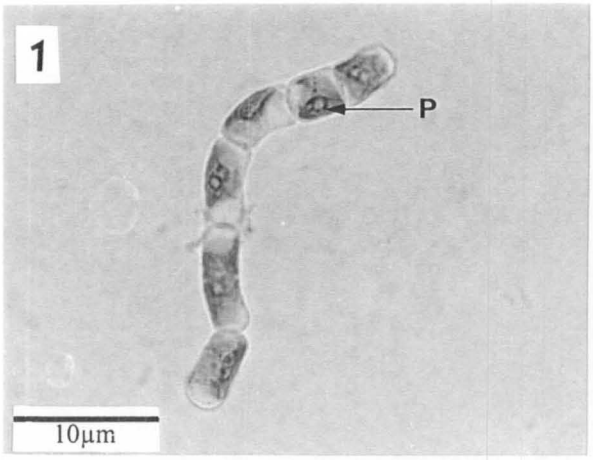


Fig. 57. *Binuclearia* and *Protoderma,* LM.

1-6. *Binuclearia* morphotype 1. 1, filament showing band-shaped chloroplast with pyrenoid (P). 2, cylindrical cells, akinetes (AK) and aplanospores (AP). 3, spherical akinete with granules. 4-6, germinating stages of akinete. 4, akinete dividing into two daughter cells. 5, akinete dividing further and developing into a germling (GM). 6, growth of germling to form filaments (FI).

7-8. *Protoderma* morphotype 1. 7, prostrate pseudoparenchymatous thallus. 8, thallus (TH) without erect branching, one cell transforming into sporangium (SP).

Scale in 1 also applies to 2-8.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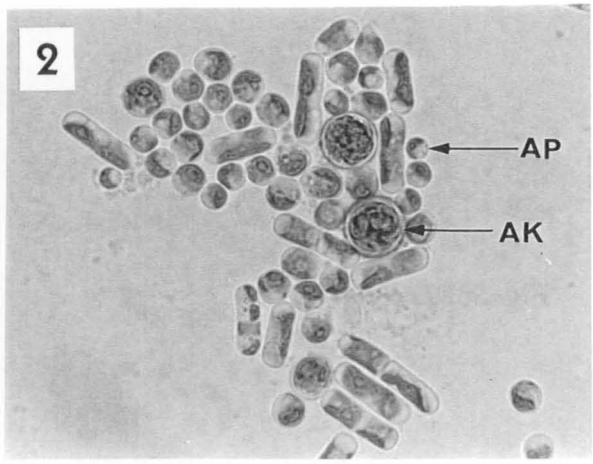
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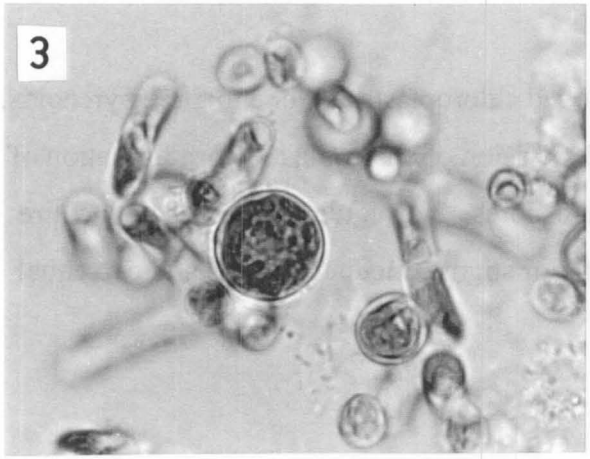
AK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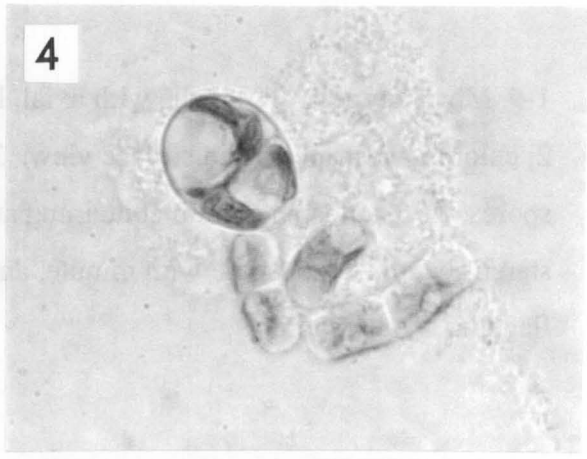
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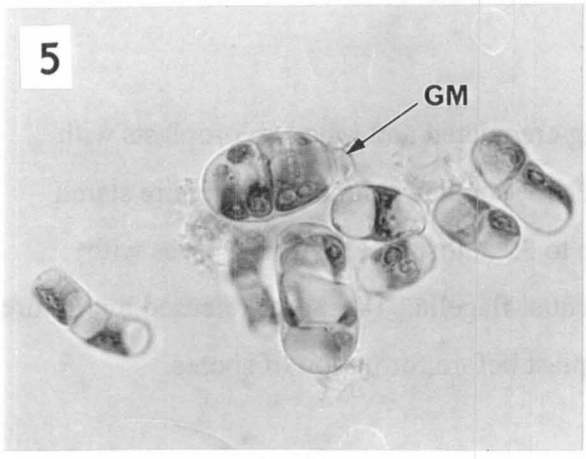
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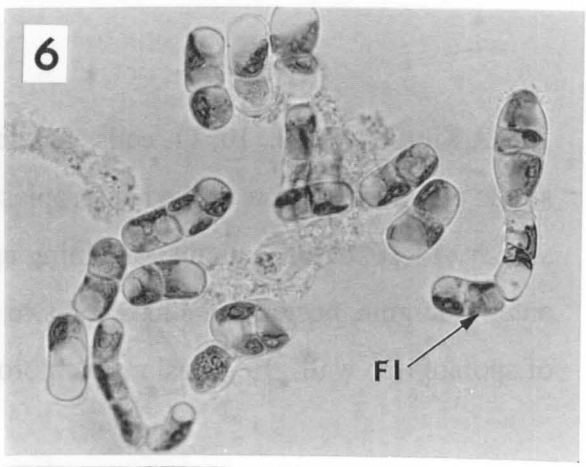
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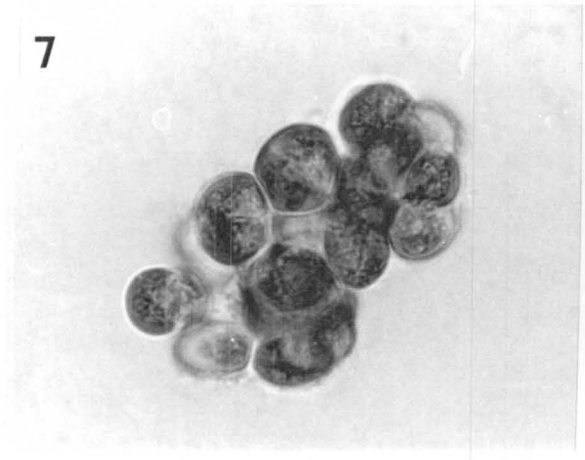
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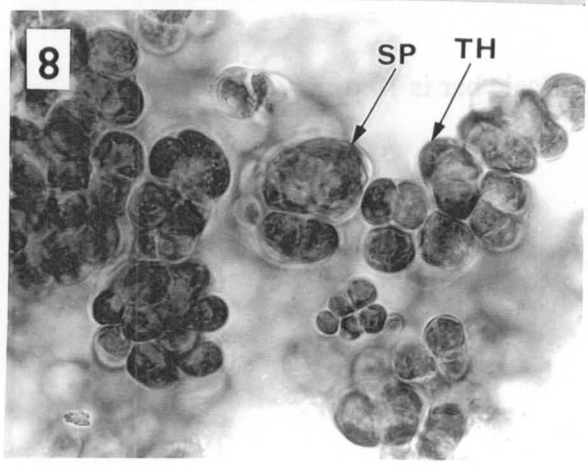
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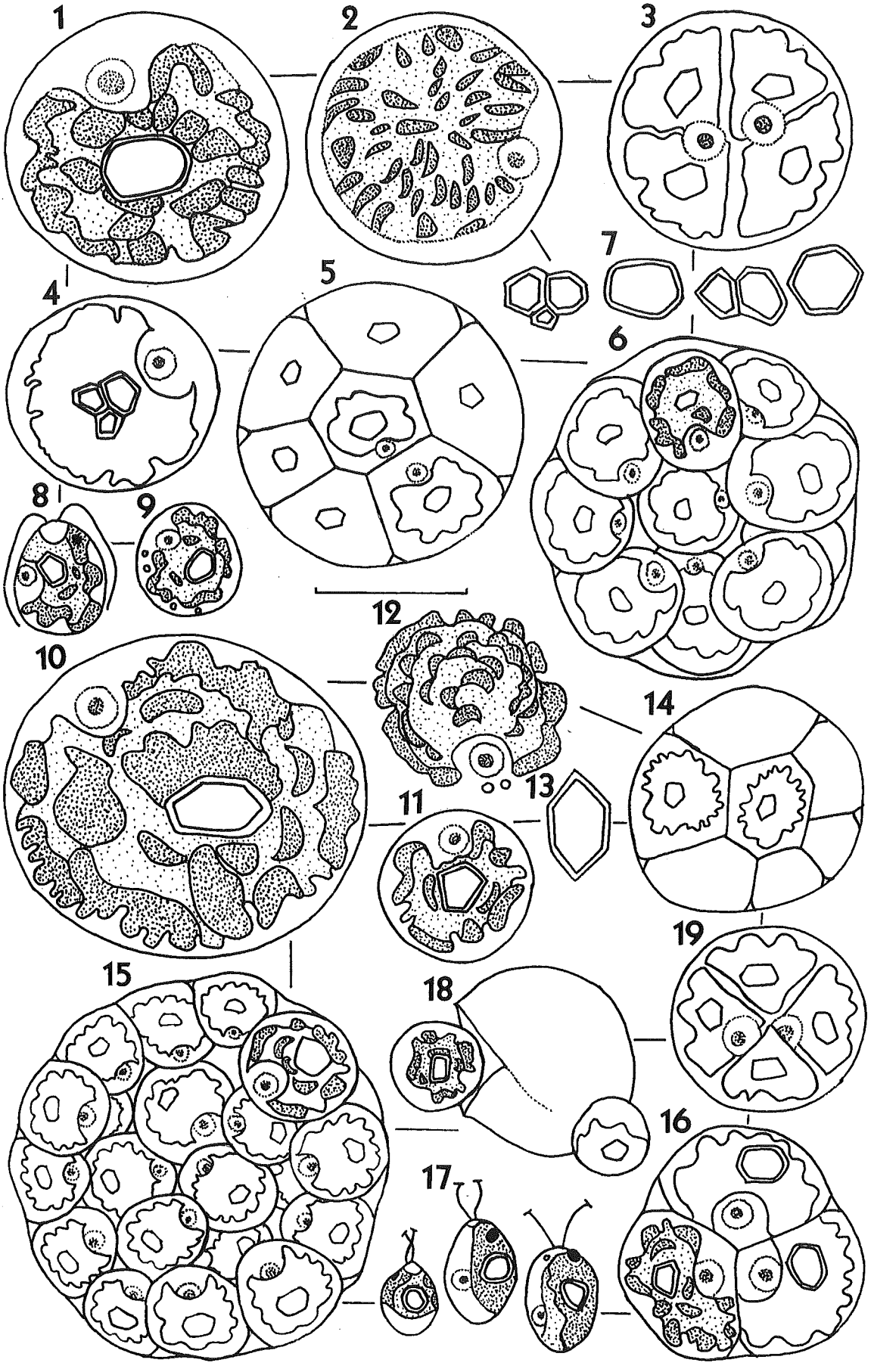
SP
TH

Fig. 58. *Trebouxia.*

1-9. Morphotype 1. 1, 4, cells with axial, lobed chloroplasts and one to three pyrenoids. 2, chloroplast stripe-like in surface view. 3, division of chloroplast before formation of spores. 5, 6, autosporangium containing autospores. 7, irregular pyrenoids with entire starch sheath. 8, zoospore with minute, anterior stigma, median nucleus and two equal flagella. 9, autospore.

10-19. Morphotype 2. 10, 11, cells showing crenulated and lobed chloroplasts with single pyrenoid. 12, crenulated chloroplast. 13, angular pyrenoid with entire starch sheath. 14-16, autosporangia containing up to 32 autospores. 17, zoospores with anterior stigma, posterior nucleus and two equal flagella. 18, spores released by rupture of sporangium wall. 19, division of chloroplast before formation of spores.

Scale bar is 10 μm.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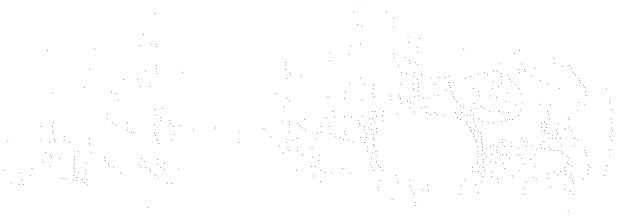
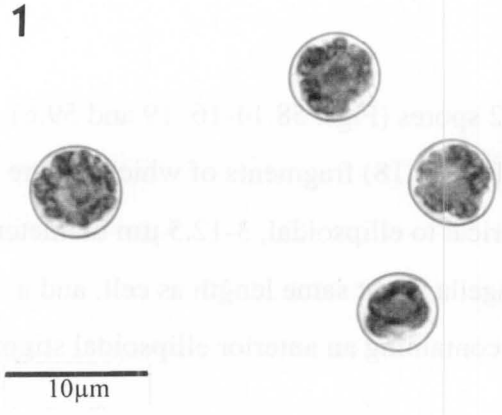


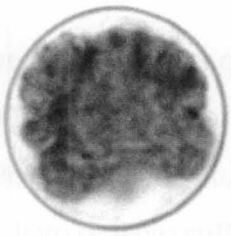
Fig. 59. *Trebouxia,* LM.

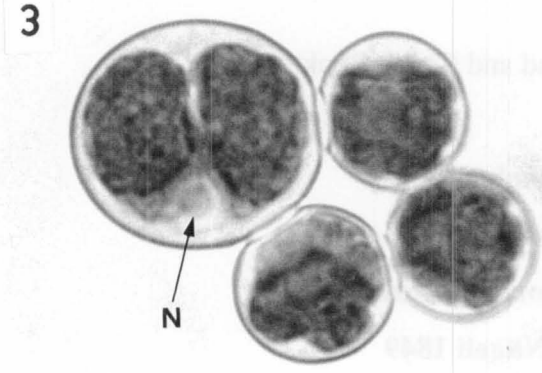
1-4. Morphotype 1. 1, young cells. 2, adult cell with axial, lobed chloroplast. 3, group of cells, one cell with divided chloroplast before formation of spores and with distinct nucleus (N). 4, autosporangium.

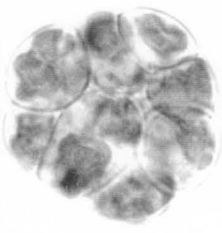
5-8. Morphotype 2. 5, group of cells each with lobed chloroplast. 6, division of chloroplast before formation of spores. 7, group of autosporangia containing 2-8 autospores. 8, a group of adherent, recently released, large autospores.

Scale in 1 also applies to 2-8.


1
10µm


2


3
N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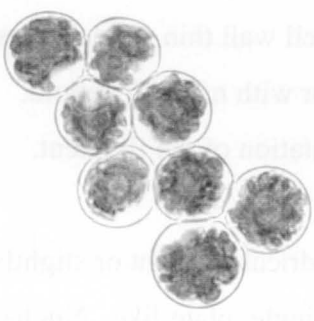
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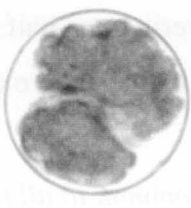
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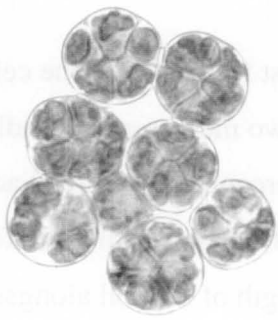
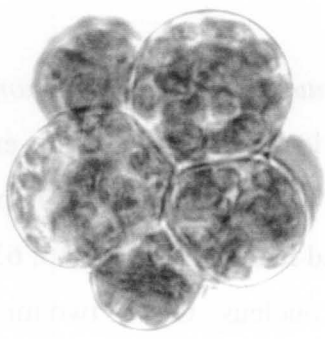

7


8

angular (Fig. 58.13), single, embedded centrally or slightly excentrically in chloroplast. Starch sheath entire.

Sporangia up to 23 μm diameter, containing 4-32 spores (Figs. 58.14-16, 19 and 59.6). Spores released by rupture of sporangium wall (Fig. 58.18) fragments of which adhere to spores. Autospores (Fig. 59.8) frequent, spherical to ellipsoidal, 3-12.5 μm diameter. Zoospores ellipsoidal to oval, 4-7 by 3-5 μm, flagella about same length as cell, and a posterior nucleus. Chloroplast with a pyrenoid, containing an anterior ellipsoidal stigma (Fig. 58.17).

Distribution: Recorded from Marie Byrd Land and Castle Rock.

Class: Charophyceae

Order: Klebsormidiales

Family: Klebsormidiaceae

***Stichococcus* Nägeli 1849**

Filaments free-living, short, readily fragmenting or cells single. Cells loosely connected, straight or curved with rounded apices. Cell wall thin. Chloroplast parietal, plate-like, covering one half or less of wall, without or with naked pyrenoid. Reproduction by vegetative cell division and fragmentation of the filament.

LM features common to all morphotypes. Cells cylindrical, straight or slightly curved with rounded apices (Fig. 60). Chloroplast parietal, single, plate-like. Nucleus lies on the side of the cell opposite the chloroplast. Reproduction by transverse vegetative cell division.

TEM features common to all morphotypes. Chloroplast fills much of the cell and runs most of its length. Chloroplast envelopes comprises two membranes. Girdle lamellae absent. Thylakoids arranged in parallel bands and interconnected with adjacent thylakoid lamellae (Figs. 62.5; 63.5, 8 and 64.2, 4, 6, 8). Golgi bodies are found on both sides of nucleus. One or two mitochondria run the length of the cell alongside the nucleus. A single peroxisome, rather difficult to discern, lies near one mitochondria.

Starch and lipid globules accumulated in the plastid stroma (Fig. 63.8). Vacuoles and small vesicles are often present.

Reproduction by transverse vegetative cell division (Figs. 62.4 and 63.3, 7).

Stichococcus morphotype 1 (S. minutus forma A)
(Figs. 60.1; 61.1 and 63.1-3)

LM. Cells in short, readily-fragmenting filaments of up to four cells. Cells 4-4.5 by 2-2.5 μm (Figs. 60.1and 61.1). Chloroplast covering about half of cell wall, lacking pyrenoid. Vacuoles occasionally present at ends of cell.
TEM. Pyrenoid absent (Figs. 63.1-3). Starch and lipid globules few, present between thylakoid lamellae.

Distribution: Recorded from Signy Island.

Stichococcus morphotype 2 (S. minutus forma B)
(Figs. 60.3; 61.4-5 and 63.4-5)

LM. Cells in short, readily-fragmenting filaments of up to five cells. Cells 6-7.5 by 2.5-3 μm (Fig. 60.3). Chloroplast covering about half of cell wall, lacking pyrenoid (Figs. 61.4-5).

TEM. Pyrenoid absent (Figs. 63.4-5). Starch and lipid globules several, present between thylakoid lamellae.

Distribution: Recorded from Marie Byrd Land, Cape Bird, Granite Harbour and Inexpressible Island.

Stichococcus morphotype 3 (S. bacillaris forma A)
(Figs. 60.4; 61.6-7 and 63.6-8)

LM. Cells in short, readily-fragmenting filaments of up to 15 cells. Cells 5.5-7 by 3.5-4 μm (Fig. 60.4). Chloroplast girdle-shaped, covering 2/3rd of cell wall, lacking pyrenoid (Figs. 61.6-7). Vacuoles often present, scattered in the cell. Culture yellowish-green.

TEM. Pyrenoid absent (Figs. 63.6, 8) or present in chloroplast (Fig. 63.7). Starch and lipid bodies several, present between thylakoid lamellae.

Distribution: Recorded from Marie Byrd Land.

Stichococcus morphotype 4 (S. bacillaris forma B)
(Figs. 60.5; 61.8-9 and 64.1-2)

LM. Cells in short, readily-fragmenting filaments of up to nine cells. Cells 8.5-9.5 by 3.5-4 μm (Fig. 60.5). Chloroplast girdle-shaped, covering 2/3rd of cell wall, lacking pyrenoid (Figs. 61.8-9).

TEM. Pyrenoid present in chloroplast (Figs. 64.1-2). Starch occasionally seen between thylakoid lamellae. Lipid bodies numerous, large, spherical, present between thylakoid lamellae.

Distribution: Recorded from Marie Byrd Land.

Stichococcus morphotype 5 (S. exiguus forma A)
(Figs. 60.6; 61.10-11 and 64.3-4)

LM. Cells in short, readily-fragmenting filaments of up to four cells. Cells short in young filaments, but very long in older ones, 10-14 by 3-3.5 μm (Fig. 60.6). Chloroplast covering most of cell wall, lacking pyrenoid (Figs. 61.10-11). Polar vacuoles often containing small oil globules. Culture yellowish-green.

TEM. Pyrenoid absent (Figs. 64.3-4). Starch and lipid bodies numerous, present between thylakoid lamellae.

Distribution: Recorded from Castle Rock.

Stichococcus morphotype 6 (S. exiguus forma B)
(Figs. 60.7; 61.12-13 and 64.5-6)

LM. Cells in short, readily-fragmenting filaments of up to 10 cells. Cells short in young filaments, but very long in older ones, 9-12.5 by 3-3.5 μm (Fig. 60.7). Chloroplast covering most of cell, lacking pyrenoid (Fig. 61.12-13). Polar vacuoles often containing small oil globules.

TEM. Pyrenoid absent (Figs. 64.5-6). Starch and lipid bodies numerous, present between thylakoid lamellae.

Distribution: Recorded from Scott Base and Marie Byrd Land.

Stichococcus morphotype 7 (S. allas forma)
(Figs. 60.8; 61.14-15 and 64.7-8)

LM. Filaments curved, up to 14 cells long. Cells 9-11 by 3.5-4 μm (Fig. 60.8). Chloroplast girdle-shaped, covering half of cell wall, lacking pyrenoid (Figs. 61.14-15). Culture yellowish-green.

TEM. Pyrenoid absent (Figs. 64.7-8). Starch and lipid bodies numerous, present between thylakoid lamellae.

Distribution: Recorded from Granite Harbour and Cape Bird.

Stichococcus morphotype 8 (cf. Stichococcus)
(Figs. 60.2; 61.2-3 and 62.1-8)

LM. Cells in short, readily-fragmenting filaments of up to four cells. Cells 5-6.5 by 3-3.5 μm (Fig. 60.2). Chloroplast covering most of cell wall. Pyrenoid immersed (Figs. 61.2-3), difficult to see with Lugol's iodine, not surrounded by starch. Starch grains numerous, scattered in chloroplast (Fig. 62.5). Culture yellowish-green.

Reproduction by autosporulation and transverse vegetative cell division. Sporangia very rare, spherical, up to 7 μm diameter, containing two to nine spores (Fig. 62.2). Spores released by rupture of sporangium wall. Sporangium wall smooth, thin. Autospores spherical, up to 3 μm diameter to ellipsoidal, 2.5-5.5 by 2-3.5 μm. Vegetative cell division frequent.

TEM. Most cells have an immersed pyrenoid which is not surrounded by starch (Fig. 62.3). Starch grains are scattered between the thylakoid lamellae. The matrix material of the pyrenoid is indistinguishable from the chloroplast stroma (Fig. 62.5).

Sporangia rarely seen, each containing two to nine spherical to ellipsoidal spores and each spore has distinct cell wall (Figs. 62.6-8). Autospores have parietal chloroplasts (Fig. 62.8) with starch and lipid globules. Rarely an autospore has a slight terminal wall thickening (Fig. 62.7). Transverse vegetative cell division is distinct and frequent (Fig. 62.4).

Distribution: Recorded from Marie Byrd Land.

Comparisons of cell length and width of the eight morphotypes of *Stichococcus* are shown in Table 3.12 and Fig. 65. Mean cell width and length varied significantly between all morphotypes. Morphotypes 2 and 1 have much narrower cell width than other morphotypes. The ranges of most strains overlapped, indicating that the strains differ little in cell width, with the exception of morphotypes 2 and 1 which do not overlap with other strains. The mean cell length of morphotype 5 is very different from

1-4 and 8 Similarly, morphotypes 6 and 7 are significantly longer than 1-3 and 8. In addition, morphotype 4 is longer than 1, 8 and 3.

Fig. 60. *Stichococcus*. Eight morphotypes of *Stichococcus* showing variation in morphology of vegetative cells.

1, morphotype 1(ISO19)

2, morphotype 8 (MB40/8)

3, morphotype 2 (599)

4, morphotype 3 (MB18/4)

5, morphotype 4 (MB7A/1)

6, morphotype 5 (G97/6)

7, morphotype 6 (756)

8, morphotype 7 (600)

Scale bar is 10 μm.

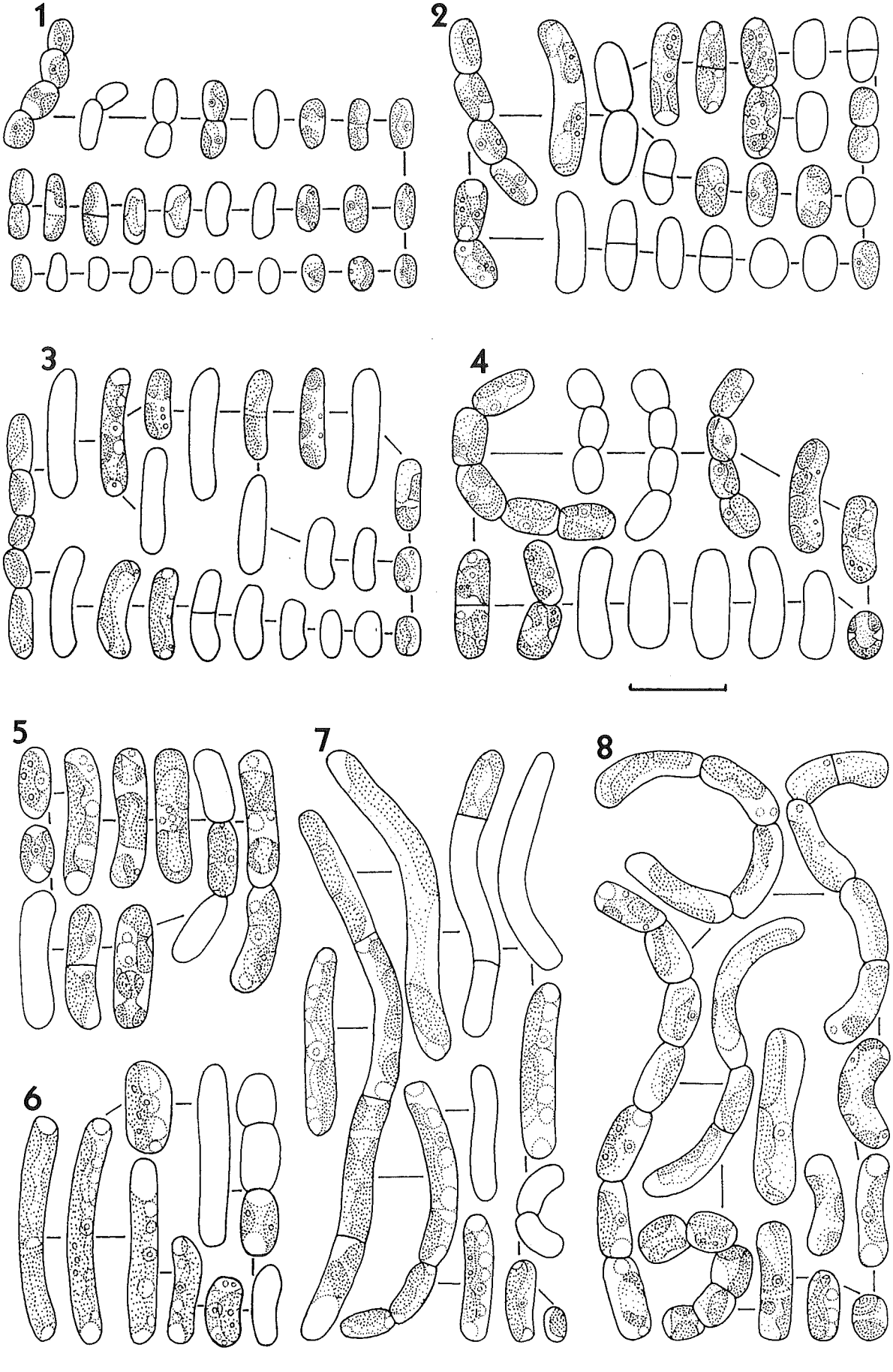

1
2
3
4
5
6
7
8

Fig. 61. Strains of *Stichococcus* showing variation in LM features of vegetative cells.

1. Morphotype 1 (ISO19), cells without a pyrenoid.

2-3. Morphotype 8 (MB40/8), cells with a naked pyrenoid (P).

4-5. Morphotype 2(599), cells without a pyrenoid.

6-7. Morphotype 3 (MB18/4), cells with a naked pyrenoid (P).

8-9. Morphotype 4 (MB7A/1), cells with a naked pyrenoid (P).

10-11. Morphotype 5 (G97/6), cylindrical and curved cells with numerous vacuoles and without a pyrenoid.

12-13. Morphotype 6 (756), cylindrical and curved cells without a pyrenoid.

14-15. Morphotype 7 (600), sigmoid filaments without a pyrenoid.

Scale in 1 also applies to 2-15.

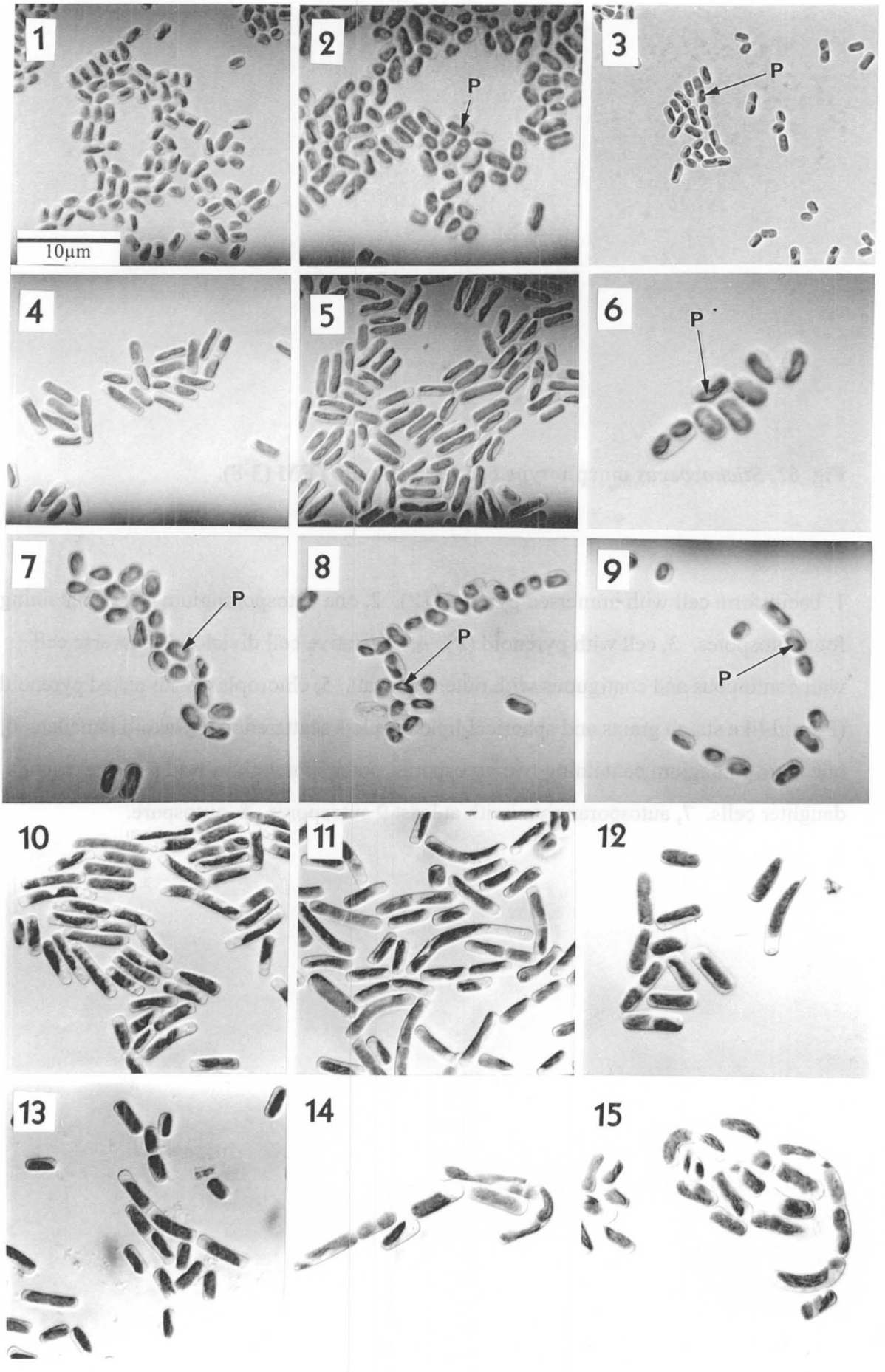
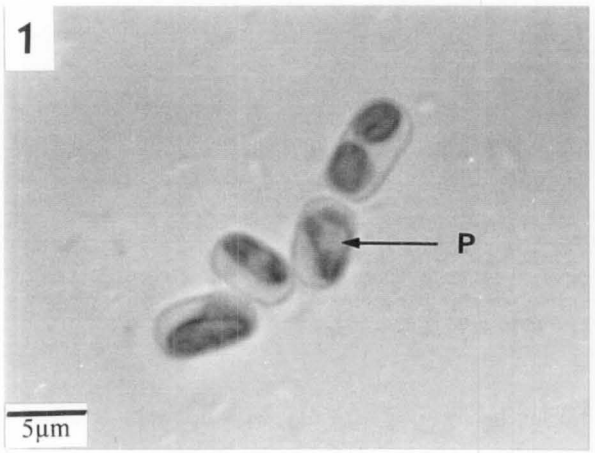
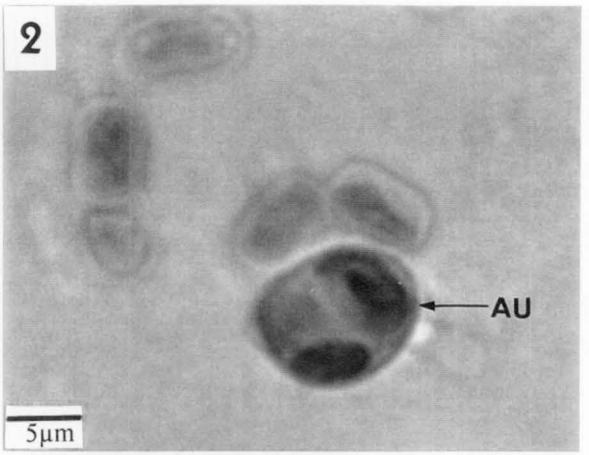
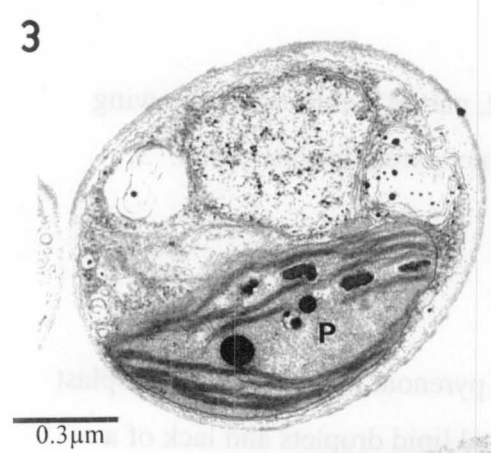

1
10μm
2
P
3
P
4
5
6
P
7
P
8
P
9
P
10
11
12
13
14
15

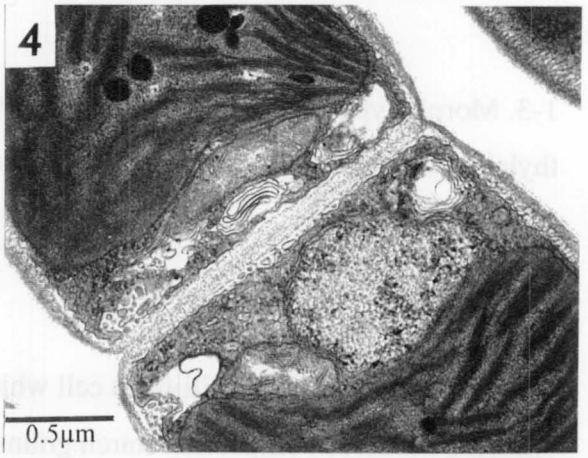
Fig. 62. *Stichococcus* morphotype 8, LM (1-2) and TEM (3-8).

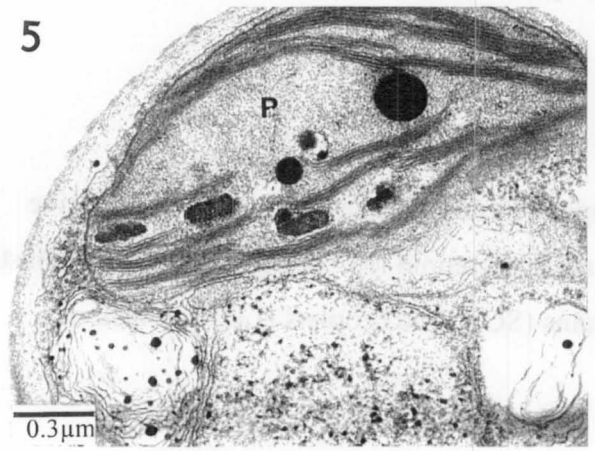
1. bacilliform cell with immersed pyrenoid (P). 2, one autosporangium (AU) containing four autospores. 3, cell with pyrenoid (P). 4, vegetative cell division, transverse cell wall continuous and contiguous with outer cell wall. 5, chloroplast with naked pyrenoid (P), rod-like starch grains and spherical lipid droplets scattered in thylakoid lamellae. 6, one autosporangium containing two autospores, outer sporangium wall (SW) surrounds daughter cells. 7, autosporangium with at least 9 autospores. 8, autospore.

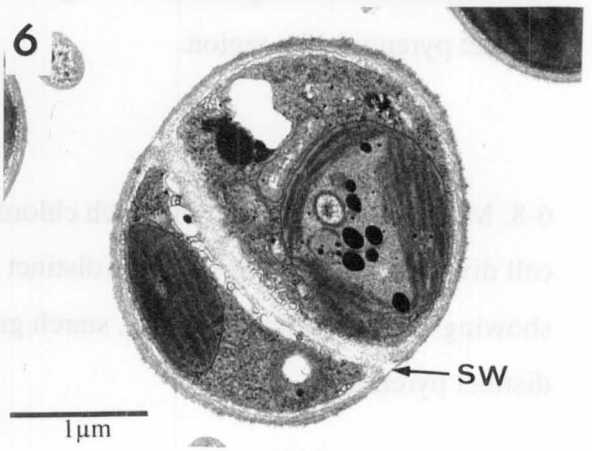

1
P
5μm

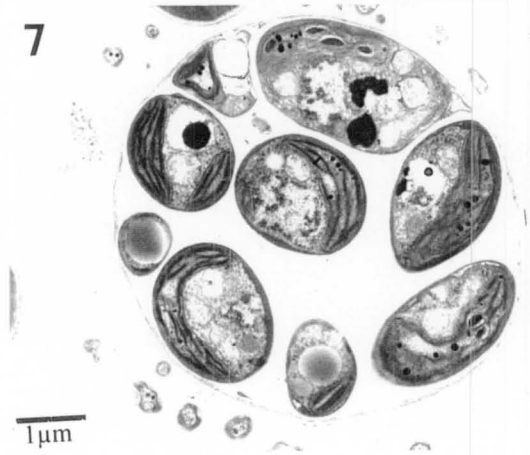

2
AU
5μm


3
P
0.3μm


4
0.5μm


5
P
0.3μm


6
SW
1μm


7
1μm

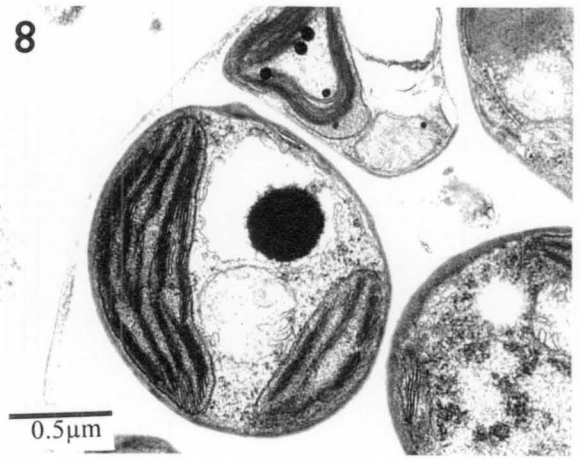

8
0.5μm

Fig. 63. *Stichococcus*, TEM.

1-3. Morphotype 1. 1, cell which lacks pyrenoid. 2, part of a chloroplast showing thylakoid lamellae, a starch grain and lack of a distinct pyrenoid-like region. 3, vegetative division.

4-5. Morphotype 2. 4, bacilliform cell which lacks pyrenoid. 5, part of a chloroplast showing numerous ellipsoidal starch grains, spherical lipid droplets and lack of a distinct pyrenoid-like region.

6-8. Morphotype 3. 6, cell in which chloroplast clearly lacks a pyrenoid. 7, vegetative cell division and one cell showing distinct naked pyrenoid (P). 8, region of a chloroplast showing thylakoid lamellae (TL), starch grains (SG), lipid droplets (LD) and lack of a distinct pyrenoid.

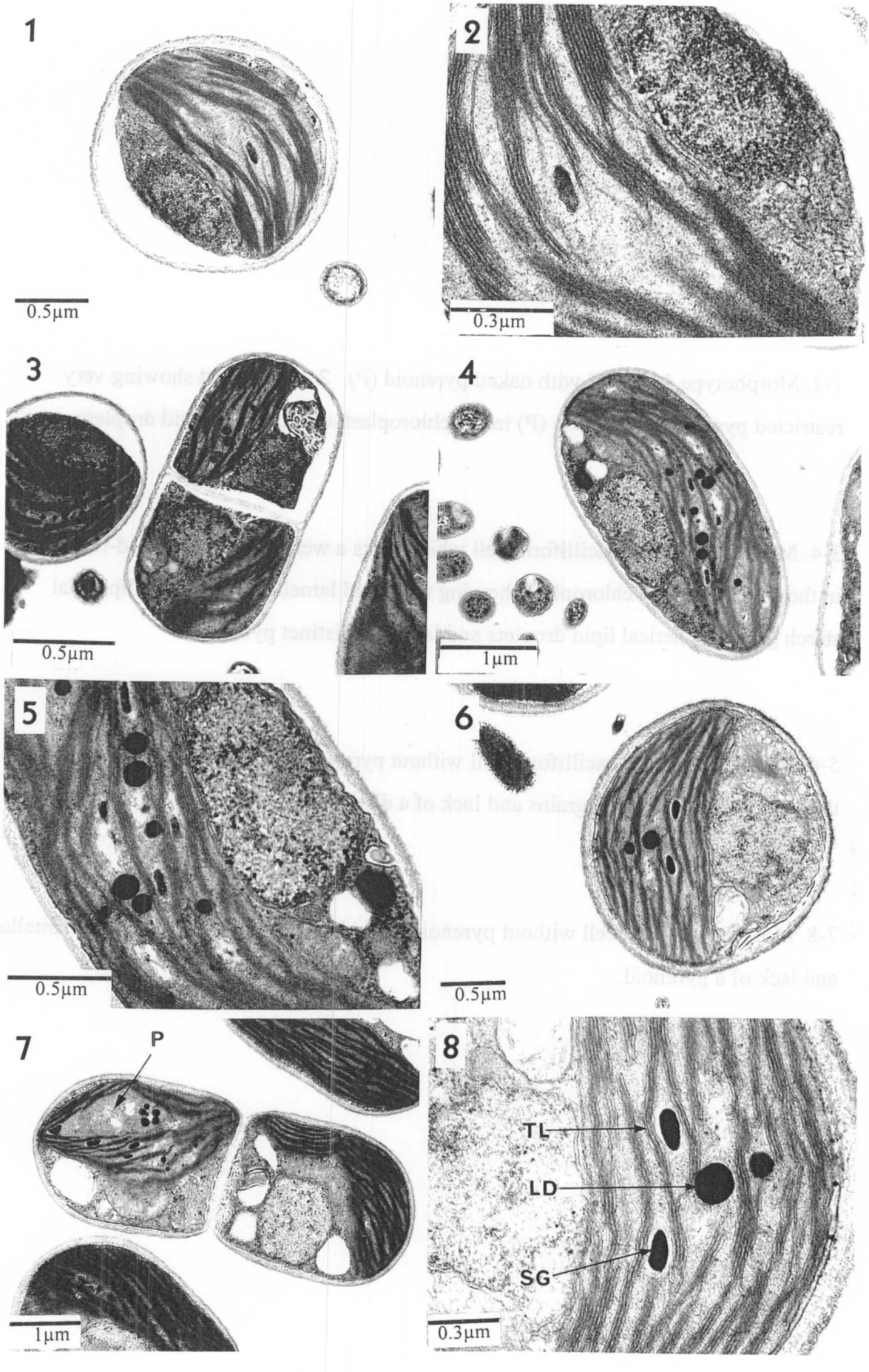

1
0.5μm
2
0.3μm
3
0.5μm
4
1μm
5
0.5μm
6
0.5μm
7
P
1μm
8
TL
LD
SG
0.3μm

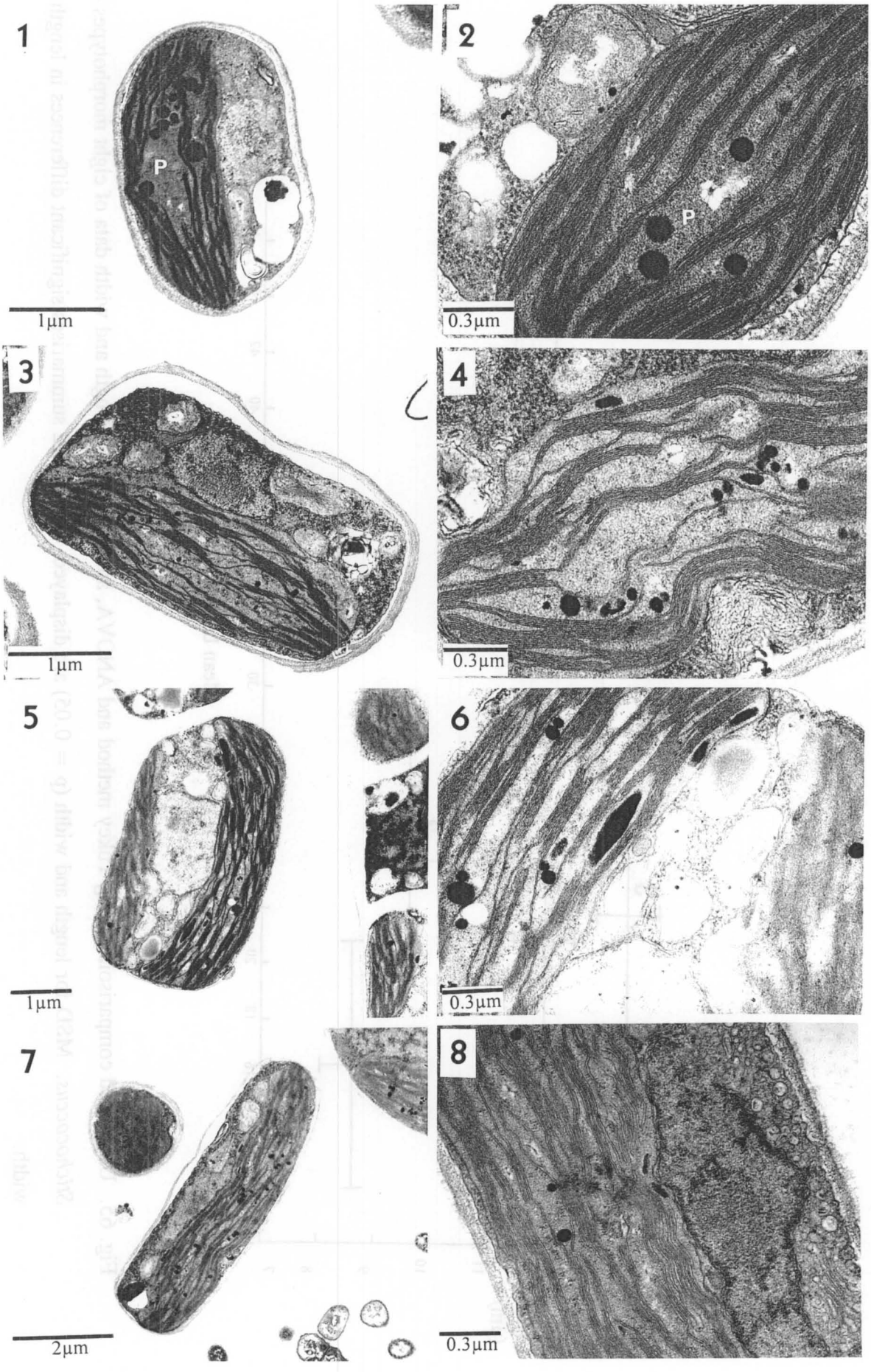
Fig. 64. *Stichococcus*, TEM.

1-2. Morphotype 4. 1, cell with naked pyrenoid (P). 2, chloroplast showing very restricted pyrenoid-like region (P) in the chloroplast and scattered lipid droplets.

3-4. Morphotype 5. 3, bacilliform cell which lacks a well-defined pyrenoid-like region in the chloroplast. 4, chloroplast showing thylakoid lamellae, scattered ellipsoidal starch grains, spherical lipid droplets and lack of a distinct pyrenoid.

5-6. Morphotype 6. 5, bacilliform cell without pyrenoid. 6, chloroplast showing thylakoid lamellae, starch grains and lack of a distinct pyrenoid.

7-8. Morphotype 7. 7, cell without pyrenoid. 8, chloroplast showing thylakoid lamellae and lack of a pyrenoid.


1
P
1μm
2
P
0.3μm
3
1μm
4
0.3μm
5
1μm
6
0.3μm
7
2μm
8
0.3μm

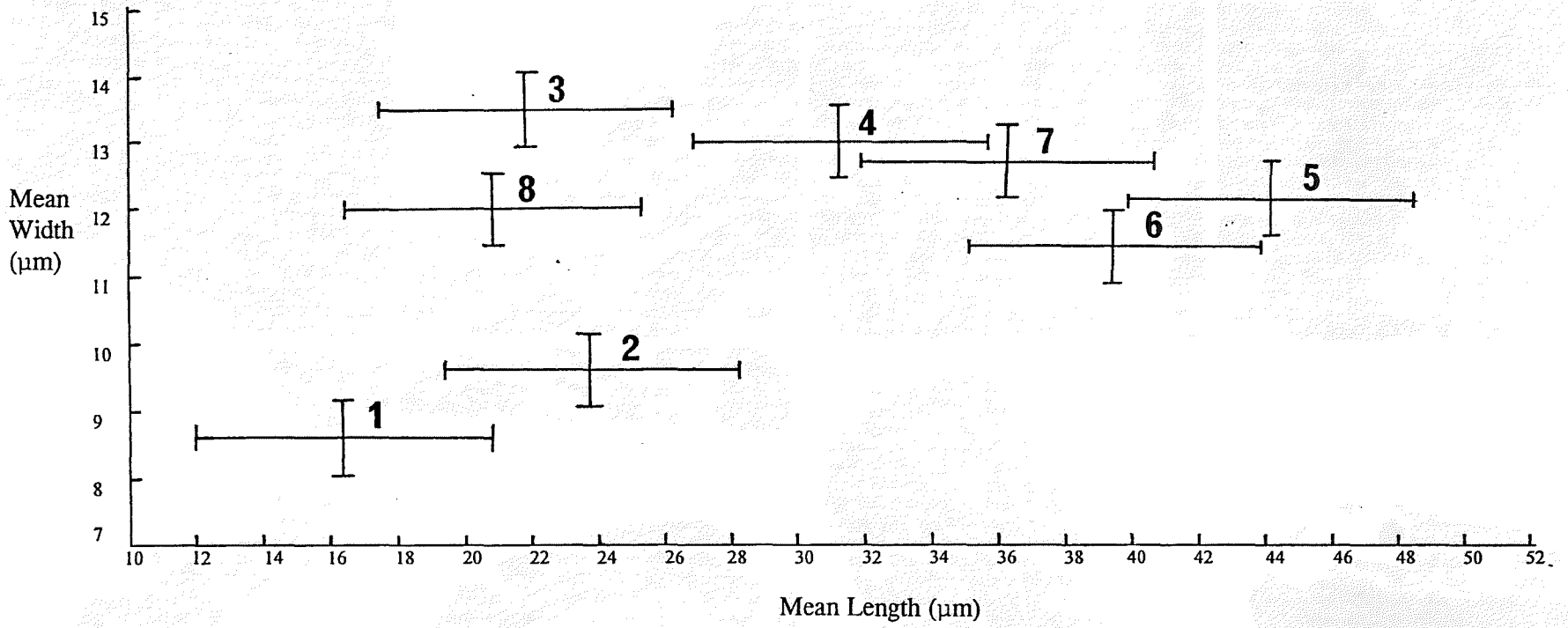


Fig. 65. Unplanned comparisons, using Tukey method and ANOVA, among means of length and width data of eight morphotypes of *Stichococcus*. MSD for length and width ($p = 0.05$) are displayed. Table 3.12 summarises significant differences in length and width.

Table. 3.12. Comparison of cell length and width of eight morphotypes of *Stichococcus*, grown under standard conditions on agarised media, using ANOVA and Tukey method for unplanned comparisons among means. Strains assigned the same letter (A-E) do not differ significantly in cell length or width at p = 0.05.

Morphotypes	Mean ± SD	Tukey grouping				
[a]Cell length						
5	44.2 ± 22.7	A				
6	39.5 ± 21.4	A	B			
7	36.3 ± 12.1	A	B			
4	31.3 ± 13.1		B	C		
2	23.9 ± 9.2			C	D	
3	21.9 ± 8.6				D	
8	20.9 ± 8.0				D	
1	16.4 ± 3.4				D	
[b]Cell width						
3	13.5 ± 1.3	A				
4	12.9 ± 1.8	A	B			
7	12.7 ± 1.7	A	B			
5	12.1 ± 2.5		B	C		
8	12.0 ± 1.2		B	C		
6	11.4 ± 1.9			C		
2	9.6 ± 1.2				D	
1	8.6 ± 1.2					E

[a]Minimum Significant Difference (MSD) = 8.44; [b]MSD = 1.03

4. DISCUSSION

4.1 Species concepts in microalgae

The purpose of this study was to investigate the diversity of Antarctic chlorophyte and xanthophycean algae from the Ross Sea region of Antarctica and to provide a taxonomic framework for future studies. An attempt has been made to provide detailed descriptive data for poorly known, unicellular xanthophyceans and unicellular and filamentous chlorophytes.

Taxonomy is a dynamic discipline and classifications are constantly changing. All sources of knowledge about organisms should be used for their classification and the delimitation of species (Komárek, 1987). However, the concept of "species" differs widely (Manhart and McCourt, 1992; Wood and Leatham, 1992).

The morphological species concept is used extensively for algae because sexual reproduction is unknown in many taxa. The implicit assumption of a morphological species concept is that every time a speciation event occurs, it will include an accompanying change in morphology (Andersen, 1998). For certain groups this appears to be the case, but not for others. The biological species concept has been applied where sexual reproduction is known, e.g. Volvocales (Coleman, 1977). In addition, morphological and biological species concepts are usually more or less congruent for macroalgae and this has been confirmed by gene sequence comparisons (e.g. Goff *et al.*, 1994). However, amongst microalgae, the data are equivocal. Overall, Andersen (1992) suggested that a consistent species concept can be found within few algal groups, e.g. *Gracilaria,* but no single species concept can be applied to all algal groups. Morphology is used explicitly or implicitly for nearly all species and therefore, the morphological species concept is used more frequently than either biological or molecular species concepts (Andersen, 1992).

Manhart and McCourt (1992) provided a review of current species concepts and considered molecular data not to be a magic bullet for species problems. Some molecular data are informative and others are misleading. One could argue that the

availability of large amounts of molecular data will unambiguously solve many problems in systematics, including the location of species boundaries. However, it is clear that molecular data are most useful when they are combined with morphological and other types of characters (e.g. Buchheim and Chapman, 1992). It is therefore unlikely that molecular data alone will change attitudes on what constitutes a species.

In this study the following species concept of Komárek (1987) is applied:
"The species comprises a set of populations between which it is impossible to find any sharp gap in any (morphological, biochemical, etc.) feature. In other words, between species there must exist some hiatus in some feature at any developmental stage of their life-cycle."

There is also a large degree of confusion amongst taxonomists as to the delimitation and application of the terms "variety" and "subspecies" (Stuessy, 1990). To avoid these confusions, the term "forma" is used here for trivial variations occurring among individuals of any population (Lawrence, 1947).

In the previous chapter the studied strains have been grouped into morphotypes, in which each morphotype differs from others in one or more significant morphological features recognised by LM and TEM. In the following sections, morphotypes are compared with known species and discussion is provided of the identity of each. Each morphotype is assigned to a species using traditional taxonomic approaches and the morphological species concept. In addition, for *Botrydiopsis, Botryochloris* and *Chlorellidium*, the results of phenetic analysis using morphological characters and isozymes are compared with these species assignments. The contribution of pigment analysis to the taxonomy of these genera is also assessed. The results of this study are then placed in the broader context of our knowledge of non-marine Antarctic algae and remaining gaps in knowledge are outlined.

4.2 Xanthophyceae

4.2.1 Comparison of Antarctic morphotypes with known species using the traditional taxonomic approach

The most prominent feature common to *Botrydiopsis, Botryochloris* and *Chlorellidium* is the presence of numerous discoidal yellow-green chloroplasts in each cell. The uniformity of structure of the zoospores is another noticeable feature. Although the zoospores may differ in size, to some extent in shape, in the presence or absence of a stigma, and in number of chloroplasts, they are invariably characterised by the presence of two unequal flagella. The three genera are also very similar in shape of vegetative cells and chloroplasts. However, in spite of structural similarities, the recognition of three genera is justified (Table. 4.1).

Vegetative division and the formation of tetrads are the distinguishing characteristics of *Chlorellidium* which separate it from *Botrydiopsis* and *Botryochloris. Botryochloris* is characterised by the possession of a distinct mucilage sheath. However, a mucilage sheath is occasionally seen in old cultures of *Botrydiopsis. Botryochloris* is also similar to *Sphaerosorus* Pascher, which differs only in the spherical shape of the colony. This is irregular in *Botryochloris*. The separation of these two genera is not convincing.

Chlorellidium has been confused with *Botrydiopsis* because of the spherical shape of adult cells in both genera (Tarapchak, 1972). *Chlorellidium* is also similar to *Chlorellidiopsis.* However, the latter differs in shape and number of chloroplasts (Table 4.1).

Ultrastructure of the Xanthophyceae has been neglected compared with many other algal groups (Hibberd, 1981; Berner, 1993). Until recently (Hibberd, 1980; Lokhorst and Segaar, 1989), very little has been published on the ultrastructure of *Botrydiopsis* and there has been no detailed account of pyrenoid structure. There are no previous reports on fine-structure of *Botryochloris* and *Chlorellidium.*

The presence of pyrenoids and their structure can be regarded not only as an interspecific, but also an intergeneric feature, e.g. *Muriella* Petersen and *Planktosphaerella* Reisigl (Komárek, 1987). However, the current concept of *Botrydiopsis* and *Chlorellidium* includes species with and without pyrenoids.

Table. 4.1. Comparison of generic characteristics of *Botrydiopsis, Botryochloris, Chlorellidium* and *Chlorellidiopsis* (Ettl, 1978).

Characteristics	*Botrydiopsis*	*Botryochloris*	*Chlorellidium*	*Chlorellidiopsis*
Adult thallus	single cell	colony with 100 or more cells surrounded by mucilage sheath	tetrad of cells	tetrad of cells
Chloroplast				
Shape	discoid, ellipsoidal or polygonal	discoid or saucer-shaped	discoid	lobed or incised
Number	1->20	1-10	2->20	1-3

Vegetative cells of *Botrydiopsis, Botryochloris* and *Chlorellidium* are almost identical in position and organisation of the nuclear envelope, chloroplast endoplasmic reticulum, thylakoids, golgi apparatus, mitochondria, lipid droplets, centrioles and genophore and are similar to other xanthophycean algae. However, minor variations occur in structural features of the chloroplast.

Chloroplasts in *Botrydiopsis, Botryochloris* and *Chlorellidium* have three-thylakoid lamellae, as do those of all other xanthophyceans studied. With the exception of the girdle lamella, the evenly spaced lamellae in all three genera normally traverse the entire plastid. The three separate non-coherent thylakoids in each lamella are clearly distinct, similar to *Botrydium granulatum* Greville (Falk and Kleinig, 1968) and *Ophiocytium majus* Nägeli (Hibberd and Leedale, 1971). Coherent thylakoids are also found in xanthophycean algae, e.g. *Tribonema viride* Pascher (Falk and Kleinig, 1968). However, both coherent and non-coherent thylakoids are found in the chloroplast of

Vaucheria De Candolle. It has been postulated that the degree of coherence is a feature no taxonomic significance and varies from fixation to fixation and cell to cell within one fixation (Evans, 1966).

Internal, semi-immersed, bulged and stalked pyrenoids are all found amongst xanthophyceans (Berner, 1993). Some species lack pyrenoids and in others they can be seen in both zoospores and vegetative cells (Hibberd, 1980). A single species from each of *Botrydiopsis* and *Chlorellidium* from Antarctica has a pyrenoid in each chloroplast.

The immersed and bulged pyrenoids of *Botrydiopsis* and *Chlorellidium* resemble those of *Pseudobumilleriopsis pyrenoidosa* (Deason, 1971b), *Mischococcus sphaerocephalus* Vischer (Hibberd and Leedale, 1971) and *Bumilleria sicula* (Massalski and Leedale, 1969). The pyrenoid is traversed by evenly spaced three-thylakoid lamellae, though these are more widely spaced than in the plastid matrix. There is no storage material surrounding the pyrenoid but lipid droplets are distributed randomly in the chloroplast matrix.

Berner (1993) considered that the complexity of thylakoid interconnections in the pyrenoid has systematic value. Series of complex interconnections formed between adjacent thylakoid bands are found in the raphidophyte *Vacuolaria virescens* Cienk. Regular interconnection is also reported for *Fucus*. Thylakoid bands with or without interconnections are found in *Botrydiopsis* and *Chlorellidium.*

The present study used hot fixation for the first time on *Botrydiopsis* and *Chlorellidium.* Where pyrenoid structure is an important taxonomic character, the use of different fixation techniques in TEM could be necessary for observing the required features.

Sixty-three species of xanthophyceans have been recorded from Antarctica up to 1998. The present investigation includes four new records of xanthophyceans.

In the following sections, each morphotype of *Botrydiopsis, Botryochloris* and *Chlorellidium* (Table 4.2) is compared with descriptions of morphospecies in the literature.

Table. 4.2. Assignment of the investigated strains of *Botrydiopsis, Botryochloris* and *Chlorellidium* to morphospecies.

Morphospecies	Morphotype	Strain No.
Botrydiopsis		
B. constricta	B1	645, 894, 829, 801, G41/6, WI/1
B. alpina	B2	895, 896, 897, 886, G12/1, 724
	B3	485
	B4	G94/1
B. arhiza	B5	836
B. callosa	B6	837, G9/8, G19/2, G24/8, G27/1, G31/5, G99/2
	B7	877, 638
	B8	864, G27/2
	B9	908
Botryochloris		
B. sp. A sp. nov.	BC1	G19/1
Chlorellidium		
C. tetrabotrys	C1	597, 757, 758, 898, G28/7, G31/6
C. sp. A sp. nov.	C2	785, 871

Botrydiopsis

The problem of species identification within *Botrydiopsis* is not a simple one. In the past, inadequate descriptions from nature and mixed cultures have contributed to confusion (Deason and Bold, 1960). It appears that the 27 strains of *Botrydiopsis* studied here can be divided into four species that are recognizable according to traditional morphological characters (Table. 4.2).

B. alpina and *B. callosa* are new records for Antarctica. Both *B. alpina* and *B. callosa* have several morphotypes which differ from each other by minor morphological features.

Vegetative division, but of a different pattern from, and lacking tetrad formation as in, *Chlorellidium,* has been found in strains of several species (Table 4.3) and is therefore considered as a generic character.

Table. 4.3. Occurrence of vegetative division in nine Antarctic morphotypes of *Botrydiopsis.*

Morphospecies	Morphotype	Vegetative division	
		14 -21 day culture	~80 day culture
B. constricta	B1	++	++
B. alpina	B2	+	-
	B3	+	+
	B4	+	-
B. arhiza	B5	+	+
B. callosa	B6	+	+
	B7	+	+
	B8	+	-
	B9	-	-

++ frequent vegetative division, + rare vegetative division and
- vegetative division absent.

In this study, bulged and immersed pyrenoids have been distinguished. Also, variation occurs in arrangement of thylakoids traversing both types of pyrenoid (Table 4.4).

In LM, a bulged pyrenoid is readily visible but an immersed pyrenoid is not. Both are distinct in TEM. More recent TEM studies of *Botrydiopsis alpina* have been concerned mainly with zoosporogenesis. Lokhorst and Segaar (1989) used ultra-rapid freeze fixation and noted that conventional fixation gave poor results.

Table. 4.4. Variation in thylakoid arrangement in both bulged and immersed pyrenoids as detected in *Botrydiopsis* and *Chlorellidium.*

Morphospecies	Morphotype	Nature of pyrenoid		Thylakoid arrangement	
		Immersed	Bulged	Interconnected between adjacent bands in the pyrenoid	Interconnected between bands at the end of or outside the pyrenoid
Botrydiopsis callosa	B6	-	+	+	-
	B7	+	-	+	-
	B8	+	-	+	+
	B9	-	+	-	+
Chlorellidium sp. A	C2	-	+	[a]+	[b]+

[a] strain 785; [b] strain 871; + present; - absent

During the present investigation it was often observed that the description of a particular species might not always fit with the type specimen. However, it was decided that this did not warrant the creation of a new species unless the variation was very distinct. For example, in the diagnosis of *B. arhiza* (Borzi, 1889), there was no mention of either the presence or absence of a stigma in the zoospores, but according to Ettl and Gärtner (1995) a stigma is lacking. Although the specimens in this study possessed a stigma, other characters were identical to those described by Borzi (1889) and Ettl and Gärtner (1995) and therefore the specimens have been assigned to *B. arhiza.*

In the traditional system of classification, only the few characteristics which are visible in LM have been used for discriminating species. These are: presence or absence of pyrenoid, shape of chloroplast, thickness of cell wall and presence or absence of stigma in zoospore. From the present study, it is clear that other features, e.g. occurrence of vegetative division, presence or absence of verrucose cell wall and nature of pyrenoid (see Appendix 3), are also important for distinguishing species. As our knowledge of

the detailed structure of each species extends, an emended species diagnosis will no doubt be needed.

In the following sections, nine morphotypes assigned to four species are compared with descriptions in the literature.

B. constricta (pages 59-66)

Morphotype B1. Strains of B1 resemble the authentic strain of *B. constricta.* Its distinguishing features are: lack of pyrenoids and a single chloroplast with a stigma in each zoospore. Also, vegetative division is frequent in both young and old cultures. This is the major characteristic separating *B. constricta* from other described species.

Broady (1976) described *B. constricta* and compared it with related species. On occasions, *B. arhiza* Borzi studied by Chadefaud (1943) produced pyriform cells, similar to *B. constricta* at an early stage of vegetative division. No mention is made of vegetative division in any species other than *B. constricta.* Ettl and Gärtner (1995) and Tschermak-Woess (1979) considered this mode of reproduction to be abnormal. However, cells exhibiting various stages of division are frequent in cultures and the process appears to be normal.

TEM confirmed the absence of pyrenoids in this species. This is the first report on ultrastructure of *B. constricta.*

This species has been recorded from a wide range of terrestrial sites at Signy Island, South Orkney Islands (Broady, 1976, 1979a). This study has extended its distribution to another five localities and shows a widespread distribution within Antarctica. *B. constricta* has been recorded only from Antarctica but this does not necessarily indicate a restricted distribution. Any apparent discontinuity may be related to lack of detailed investigations of similar habitats on other continents.

B. alpina (pages 67-83)

Morphotype B2. This is similar to the authentic strain of *B. alpina.* The distinguishing characteristics are: absence of mucilage sheath and pyrenoid, long flagellum of zoospore shorter than in *B. constricta*, and absence of vegetative division in old cultures but its rare occurrence in young cultures. Unlike *B. arhiza,* the zoospore has a stigma, and unlike *B. anglica* Fritsch and John, the small flagellum is usually very difficult to see. There exist differing reports of the number of chloroplasts in zoospores of *B. alpina.* Vischer (1945) reported one to two, Reisigl (1964) two to three and Tschermak-Woess (1979) two. In B2, the zoospore has one or two chloroplasts which is similar to *B. intercedens* Pascher. However, adult cells of B2 are smaller (up to 36 μm diameter) than both *B. intercedens* (up to 70 μm diameter) and *B. arhiza* (up to 80 μm diameter). Pascher (1937) described cells of *B. intercedens* up to 60-70 μm in diameter (p. 389), Tschermak-Woess (1979) described cells of *B. intercedens* up to 16-18 μm, exceptionally up to 32 μm (p. 191). Vischer (1945) also reported that in the same culture condition, cell sizes of *B. intercedens* were usually smaller than *B. alpina* (p. 487). Cell sizes of *B. intercedens* might be variable among strains or culture conditions.

In B2, the zoospore has only one stigma even when two chloroplasts are present. This agrees with the observation of Tschermak-Woess (1979) who reported in detail on zoospore formation. In contrast, Nakano and Handa (1990) reported that when two chloroplasts were present in *B. alpina* both contained a stigma.

Vischer (1945) described *B. alpina* from alpine soil, Switzerland. Ettl and Gärtner (1995) considered this species to be an ecotype of *B. intercedens.* However, Tschermak-Woess (1979) regarded *B. alpina* as a distinct species, an opinion with which Nakano and Handa (1990) agreed.

Strain 895 (= CCAP 806/2), isolated by Pringsheim was misidentified as *B. arhiza.* This strain has stigmate zoospores and a smooth cell wall whereas astigmate zoospores and a verrucose wall are the distinguishing features of *B. arhiza.*

B. alpina occurs in soil (Vischer, 1945; Reisigl, 1964) and on bark (Nakano and Handa, 1990) as free-living cells and also occurs as a lichen photobiont (Tschermak-Woess, 1979). It has not been recorded previously from Antarctica.

Morphotype B3. This is similar to B2 except for its smaller sporangia and presence of rare vegetative division in young and old cultures. Zoospores of B3 have a single chloroplast with a stigma, but all other features identify it as *B. alpina.*

Morphotype B4. This is similar to B2 and B3 but differs slightly in the following characteristics. The sporangium is larger (up to 41 μm maximum diameter) in B4 than in B2 (up to 36 μm maximum diameter). A lens-shaped thickening of the cell wall is absent in B4 but occasionally present in B2 and B3. Vegetative division is rare and seen only in young cultures in B2 and B4, but in B3 it is present in young and old cultures. Zoospores contain a single chloroplast, unlike B2 in which there are one or two chloroplasts. It is assigned as *B. alpina* forma.

B. arhiza (pages 75, 84-90)

Morphotype B5. This resembles *B. arhiza* but differs in the smaller zoospores and their possession of a stigma. The zoospores are up to 4 μm long whereas those of *B. arhiza* are up to 12 μm long (Borzi, 1889). The main characteristics of this species are the verrucose wall, absence of a pyrenoid and presence of two chloroplasts in the zoospore. These features separate this from other species. B5 is assigned as *B. arhiza* forma.

B. arhiza was first described by Borzi (1889). Deason and Bold (1960), in an examination of a strain of *B. arhiza* isolated from soil, illustrated an almost cylindrical cell which is slightly constricted centrally (their fig.123). They do not describe any development of this constriction into a vegetative division, as seen in strains examined in this study, despite observing the alga in culture. They state that cells do not exceed 35 μm diameter (in B5 cells attain 43.5 μm diameter) but according to Prescott (1962) adult cells can attain 70 μm. *B. arhiza* has a very broad ecological range. For instance, it has been found in standing waters, stagnant moorland pools, soils and on tree bark. It

is recorded from Vestfold Hills (Broady, 1986) and Ongul Island (Akiyama, 1968) without detailed descriptions of specimens.

B. callosa (pages 90-113)

Morphotype B6. This can be assigned to *B. callosa* Trenkwalder. The main distinguishing characteristics of this species are presence of a bulged pyrenoid visible by LM and rare vegetative division in young and old cultures.

This is the first report on ultrastructure of *B. callosa.* TEM confirmed the presence of a pyrenoid and showed that the pyrenoid matrix is penetrated by widely spaced thylakoid lamellae.

Trenkwalder (1975) was the first to describe species of *Botrydiopsis* which possess pyrenoids. *B. pyrenoidosa* and *B. callosa* were found in soil samples of pine woods in the Brixen region, Italy. They have not been reported since. This apparently disjunct distribution probably simply reflects lack of sufficiently detailed investigations in other regions. *B. pyrenoidosa* differs from *B. callosa* in that the zoospore with a single flagellum, lacks a stigma and the cell wall of vegetative cell does not thicken with age.

This is the first record of *B. callosa* from Antarctica.

Morphotype B7. This resembles B6, but differs in the nature of the pyrenoid which is immersed and does not bulge distinctly from the chloroplast as in B6. Other features are identical with *B. callosa.* These slight differences suggest B7 is a form of *B. callosa.*

Morphotype B8. This is similar to B6 and B7, but differs by the following characteristics: pyrenoid immersed as in B7 but not B6; vegetative division rarely seen in young cultures only unlike B6 and B7 in which it is rarely seen in both young and old cultures. In B8, the only thylakoid lamellae which are interconnected are those which traverse the pyrenoid. The interconnections occur in the pyrenoid and also in those lengths of these lamellae which lie outside the pyrenoid. Within the pyrenoid these

lamellae are parallel and widely spaced. Because these differences are minor, B8 is regarded as a form of *B. callosa.*

Morphotype B9. This is close to B6-8, but differs in the following characteristics: the pyrenoid is bulged but less distinctly than in B6; vegetative division is absent; vegetative cells (up to 22 μm diameter) are smaller than B7 (up to 31 μm diameter); aplanospores (up to 5 μm diameter) are smaller than B6 (up to 12.5 μm diameter), B7 (up to 12.5 μm diameter) and B8 (up to 19 μm diameter); thylakoid lamellae in B6 and B7 are frequently interconnected in the pyrenoid but not in B9 in which they are interconnected only at the end of the pyrenoid; these interconnections do not occur outside the pyrenoid in B9. These slight differences suggest that B9 is also a form of *B. callosa.*

Botryochloris

The key characteristics which distinguish species of *Botryochloris* are the size of vegetative cells, the number of chloroplasts and the presence or absence of a stigma in zoospores. Three of the four known species, *B. minima* Pascher, *B. cumulata* and *B. simplex* Pascher have been recorded from soil (Ettl and Gärtner, 1995). Only *B. minima* has been recorded from Antarctica but no reproductive details are provided (Starmach, 1995). The description of the vegetative stage of his Antarctic specimens fits the genus, but zoospores were not observed. However, zoospores of *B. minima* contain a prominent stigma whereas this is lacking in zoospores of *B. simplex* (Ettl, 1978). The species designation of Antarctic specimens requires confirmation.

In this study, a new species, *Botryochloris* sp. A, has been described and compared with known species.

Botryochloris sp. A sp. nov. (pages 114, 116-117, 126-127)

Morphotype BC1. This can be assigned to *Botryochloris* because of the presence of a mucilage sheath and the lack of a pyrenoid (Ettl and Gärtner, 1995). BC1 is close to *B.*

cumulata Pascher because of the similar size of vegetative cells, but differs in that BC1 has 2 to 10 chloroplasts and 2 to 22 spores while *B. cumulata* has only 2 to 4 chloroplasts and 2 to 4 spores (Ettl and Gärtner, 1995). BC1 is also close to *B. chlorellidiopsis* Pascher. Both have more than four chloroplasts in adult cells. However, in *B. chlorellidiopsis,* the mode of reproduction is by aplanospores alone which clearly differs from BC1 which propagates by aplanospores and zoospores. *B. minima* has two chloroplasts and smaller cells (up to 7 μm diameter) than BC1. Zoospores of BC1 contain a stigma whereas this is lacking in zoospores of *B. simplex.*

BC1 is distinctly different from the four known species and is assigned as a new species, *Botryochloris* sp. A.

> Diagnosis: "Adult cells spherical to occasionally ellipsoidal, 6.5-15 μm diameter, aggregated within mucilage sheath. Chloroplasts up to 10, parietal, discoidal to polygonal, lacking pyrenoid. Reproduction by aplanospores and zoospores, up to 22 spores in a sporangium. Aplanospores mostly spherical, occasionally ellipsoidal, 2.5- 7 μm diameter, with one to two chloroplasts. Zoospores 4-7 μm by 2.5-4.5 μm, naked, fusiform to amoeboid, unequally biflagellate, with a hyaline posterior region and a single chloroplast containing an anterior stigma."
>
> Holotype: Figs. 25.1-8 and 29.16-25.
>
> Locality: Isolated from dark green moss cushions in dripping meltwater below rock overhang, Granite Harbour, southern Victoria Land, Antarctica ($77^0 01'$S, $162^0 30'$E), January, 1994.

Chlorellidium

Two species of *Chlorellidium* are recorded in the literature. *C. tetrabotrys* and *C. astigmatum* Schwarz (Ettl, 1978; Ettl and Gärtner, 1995) are both found in soil. The major feature separating them is the absence of a stigma in zoospores of the latter but its presence in the former.

Unidentified *Chlorellidium* species have been recorded from soil samples collected at Vestfold Hills (Broady, 1987a) and from soil and snow drifts at Scott Base (Broady and Smith, 1994).

In the following sections, two morphotypes assigned to two species are compared with the literature.

C. tetrabotrys (pages 118-119, 120-125)

Morphotype C1. This resembles the authentic culture of *C. tetrabotrys* Vischer and Pascher (strain SAG 811/1). The main features which distinguish it from C2 are absence of pyrenoid and larger vegetative cells (up to 33 μm diameter).

There have been several LM studies on *Chlorellidium* (Pascher, 1939; Ettl,1978; Ettl and Gärtner, 1995; Tschermak-Woess, 1979), but no reports on ultrastructure. TEM confirmed the absence of a pyrenoid and other ultrastructural features are typical of xanthophycean algae. Thylakoids occasionally cross from one lamella to another. A girdle lamella is a characteristic feature of the chloroplast in most members of the Xanthophyceae as well as other algal groups (Berner, 1993). It is absent in some xanthophyceans, e.g. *Pseudobumilleriopsis pyrenoidosa* Deason and Bold (Deason, 1971b) and *Bumilleria sicula* Borzi (Massalski and Leedale, 1969). During the present study, all strains possessed a three-thylakoid girdle lamella except the two strains of *C. tetrabotrys* (strain no. 898 and 597) examined by TEM in which it is reduced from three to one thylakoid along short lengths. In xanthophyceans, no report has been made on the reduction of number of thylakoids along short lengths of the girdle lamella as found in this alga.

C. tetrabotrys was originally isolated from greenhouse soil in Central Europe (Vischer, 1937). The only other probable record of this species is that of Pascher (1939) who describes a very similar alga which has been linked to *C. tetrabotrys* by Tarapchak (1972).

In Antarctica, this alga is recorded from only Vestfold Hills as an epiphyte on moss (Broady, 1986), but no detailed description was provided.

Chlorellidium sp. A sp. nov. (pages 119, 126-131)

Morphotype C2. The main distinguishing characteristic of C2 is the presence of a pyrenoid. This has not been described previously for *Chlorellidium* and hence it is erected as a new species *Chlorellidium* sp. A. Also, variation occurs in arrangement of thylakoid traversing the pyrenoid (Table 4.4).

> Diagnosis: "Single adult cell spherical to ellipsoidal, up to 21.5 μm diameter, but mostly about 12.5 μm diameter. Chloroplasts more than 20 in adult cells, discoidal to polygonal, each with a pyrenoid projecting as a bulge from the inner face. Reproduction by aplanospores, zoospores and vegetative division. Sporangia spherical to ellipsoidal, up to 33 μm diameter, containing two to numerous (>20) spores, two and four spores frequent. Spores released by rupture of sporangium wall. Aplanospores spherical to ellipsoidal, 2.5- 12 μm diameter, mostly with one to two chloroplasts, occasionally up to six. Zoospores 3.5-7 μm by 2.5-5 μm, sub-spherical to pyriform to amoeboid, unequally biflagellate, with single chloroplast containing an anterior stigma. Vegetative division forming tetrads and tetrad complexes."
>
> Holotype: Figs. 29.1-15; 30.1-8 and 31.1-6.
>
> Locality: Isolated from unknown soil, Christchurch, New Zealand ($43^0 30'$S, $172^0 30'$E), 1989 and from traces of soil on fresh vegetables brought to Scott Base, Antarctica ($77^0 50'$S, $166^0 48'$E) from New Zealand, 1991.

4.2.2 Phenetic analysis

The primary factor in the selection of a method of data analysis was objectivity. A

technique was required that would minimise the degree of subjectivity, one of the major sources of bias in taxonomic analysis in the past. The two methods of analysis that best fitted this criterion were numerical phenetics (Sokal and Sneath, 1963) and cladistics (proposed by Hennig, 1965), otherwise known as phylogenetic systematics or parsimony analysis.

Cladistic analysis was considered unsuitable as one of the requirements is the polarisation of character states, categorising them as primitive or advanced (Hennig, 1965). The most efficient method of achieving this is by means of "outgroup" comparison, i.e. comparison of the characters with those of a group known to be closely related to the taxa under investigation (Stevens, 1980). Where the outgroup is unclear, as is the case with *Botrydiopsis, Botryochloris* and *Chlorellidium,* then "ingroup" analysis is possible, the most common character states within the group under investigation being taken as primitive. However, Stevens (1980) showed this method to be questionable, particularly when applied to small groups. In the absence of a reliable outgroup, it seemed safer not to use a cladistic analysis at this stage (Stuessy, 1990). Therefore, numerical phenetics was utilised. Numerical phenetics does not take evolutionary lineage into account, and may easily be applied to groups of uncertain ancestry (Sokal and Sneath, 1963).

In Unweighted Pair Group Method with Arithmetic averages (UPGMA), two strains (operational taxonomic units, OTUs) with the highest genetic identity values are clustered first. The remaining strains are then linked in cyclic fashion at the average values of their similarity coefficients relative to already clustered strains. UPGMA has been used in other groups to infer evolutionary pathways (Nei, 1975), but in the present study UPGMA was used only to reveal the relative similarity of the strains.

Sneath and Sokal (1973) believed that phenetic analysis would allow greater discrimination and sensitivity in delimiting taxa, leading to a more accurate classification and better keys than those obtained using conventional methods. Importantly, to be placed in a group, a taxon requires the majority of diagnostic features possessed by the group. Strains identified as the same species usually formed groups at lower levels of dissimilarity.

a) Phenetic analysis of morphological data

Morphological variation in *Botrydiopsis* and *Chlorellidium* has not been studied previously using phenetic analysis. However, this technique has been used in higher plants (e.g., Ward, 1993a, b). In higher plants, numerical phenetics showed that, on the basis of overall similarity, boundaries of species are clear-cut with few exceptions. For instance, Ward (1993a, b) distinguished 21 species of *Raoulia* but not *R. mamillaris* and *R. bryoides* and suggested that the former species might be better classified as a subspecies of the latter. However, all species are clustered at 0.35 dissimilarity level.

Cluster analysis of morphotypes of *Botrydiopsis* (Fig. 66) grouped different morphotypes of *B. callosa* and *B. alpina* into widely separated clusters at greater than 0.4 dissimilarity. The similarity values (S_j) between these morphotypes ranges from 0.63 to 0.84.

Two morphotypes of *B. callosa,* B9 and B7 form a distinct cluster at 0.29 dissimilarity level and widely separated from morphotypes B6 and B8 of the same species.

The greatest similarity was between *B. constricta* (B1) and *B. alpina* (B2) which clustered at 0.22 dissimilarity level. *B. alpina* B2 shares a number of vegetative and reproductive features with *B. constricta.* Both have a high S_j value (0.87) which supports this similarity (Table 3.3).

In contrast, the greatest dissimilarity was between *B. arhiza* and all other species which grouped at 0.5 dissimilarity level. The S_j value between *B. arhiza* and remaining species ranges from 0.53 to 0.75. *B. arhiza* is clearly a distinct species in the dendrogram.

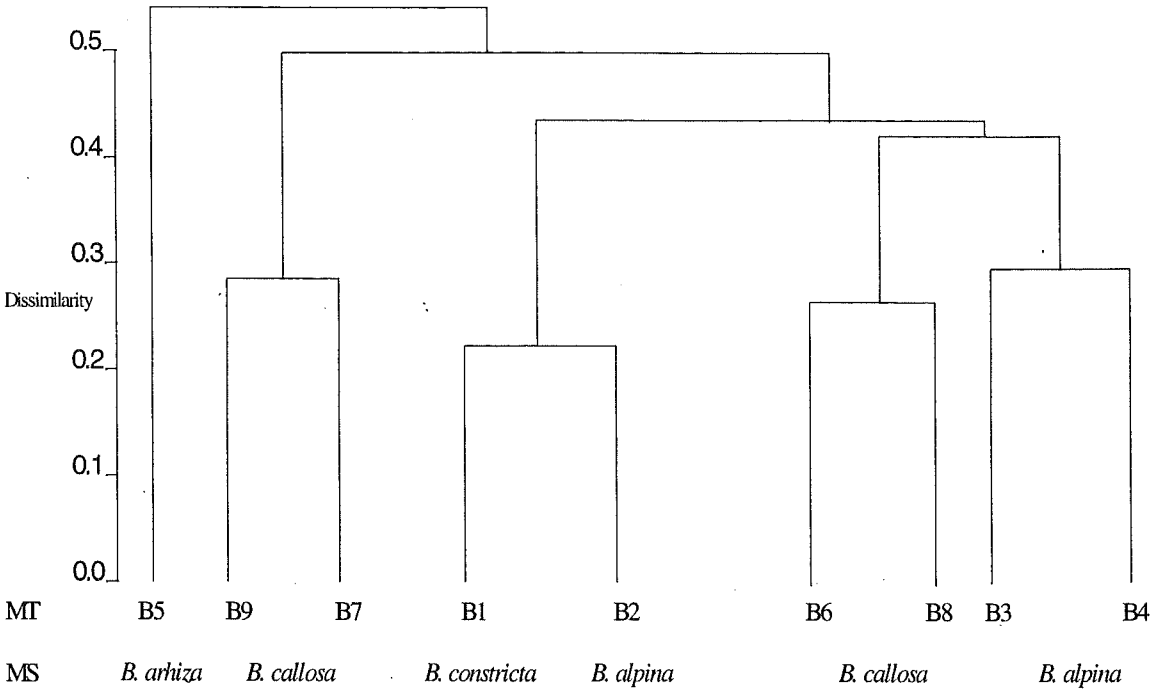


Fig. 66. Cluster analysis dendrogram using similarity coefficient with average linkage based on morphological characteristics of 27 strains of *Botrydiopsis* assigned to morphotypes (MT) B1-B9 which have been assigned to four morphospecies (MS).

Of the four morphospecies of *Botrydiopsis*, *B. callosa* and *B. alpina* are heterogeneous. This requires further investigation using molecular techniques, e.g. DNA/DNA hybridization for detecting variation within species. Previously this technique has been successfully used in *Chlorella* to detect interspecific variation (Huss *et al.*, 1986).

Cluster analysis of *Chlorellidium* (Fig. 67) showed *C. tetrabotrys* and *C.* sp. A, to group at 0.22 dissimilarity level. Both species have high S_j values (0.87).

The results of the phenetic study of morphological data show that species recognised in the traditional system are not distinct from one another in terms of overall

morphological resemblance. Phenetic analysis is not congruent with species designations in the traditional system of classification.

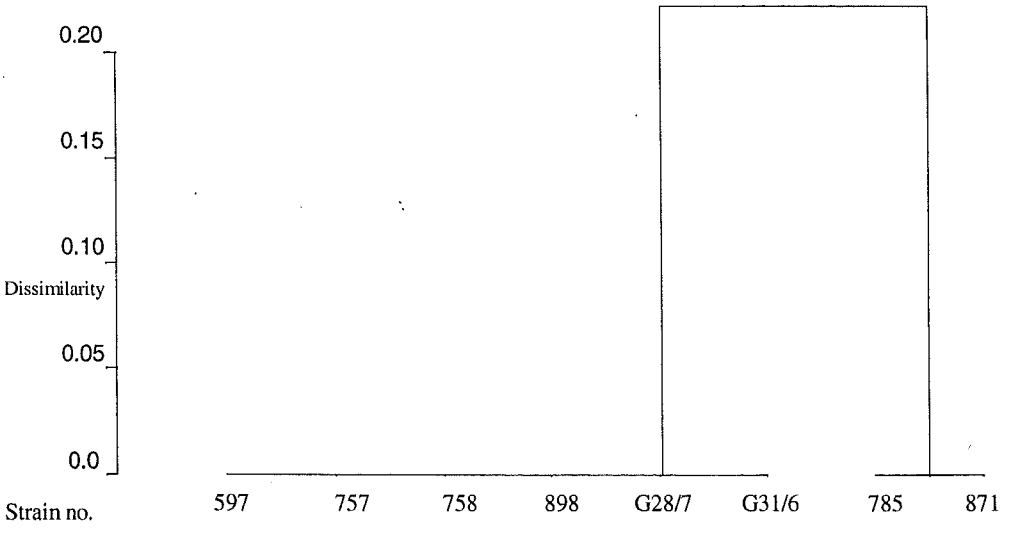


Fig. 67. Cluster analysis dendrogram using similarity coefficient with average linkage based on morphological characteristics of eight strains of *Chlorellidium* assigned to two morphotypes (C1-C2) which have been assigned to two morphospecies.

The traditional system of classification is based on unequal weighting of characters. Vegetative division is the only distinguishing feature which separates *Chlorellidium* from *Botrydiopsis.* These genera still require clarification of their distinguishing features and molecular genetical approaches are needed to improve resolution.

b) Phenetic analysis of isozyme patterns

The utility of isozymes as characters in phylogenetic investigations may be severely limited, depending on the taxonomic group or level (Murphy, 1988). Enzymes have

usually proved to be a useful tool for defining species boundaries and investigating population structure (Benzie *et al.,* 1997) but not for determining phylogenetic relationships between genera. However, intergeneric phylogeny, i.e. evolutionary relationships among genera, is also documented, e.g. in birds (Johnson *et al.,* 1988), snakes (Murphy, 1988), and rodents and their ectoparasites (Hafner and Nadler, 1988). Most studies have focused on intrageneric relationships and these are only informative when the individual loci are analysed as discrete characters (Murphy, 1993). The methods of data analysis are varied and highly controversial. Undoubtedly, these are still in a relatively early stage of refinement and remain to be developed (Murphy *et al.*, 1996).

In this study, electrophoresis has been investigated as a means of distinguishing *Botrydiopsis, Botryochloris* and *Chlorellidium* and of providing insight into genetic variation within a particular morphotype.

It is clear (Fig. 39) that *Botrydiopsis, Botryochloris* and *Chlorellidium* cannot be distinguished by electrophoresis. This result is consistent with several studies on other algae (e.g. Benzie *et al.*, 1997; Hayhome and Pfiester, 1983; Murphy and Guillard, 1976; Chinain *et al.*, 1997; Gallagher, 1980). In most cases, the technique has been used at subspecies, intra- and interspecific ranks and has been used only with limited success to investigate the taxonomy of algae above species level (Soudek and Robinson, 1983). However, in bryophytes (Melick *et al.*, 1994), higher plants (Ward, 1993c) and insects (Bush and Kitto, 1978), isozyme analysis shows genetic differences among closely related genera.

Strains of a particular morphospecies within a genus occur in separate clusters. For example, strains from *B. alpina* are scattered over the dendrogram.

Isozyme electrophoresis has shown high intraspecific genetic variability in several algae, e.g. *Pandorina morum* Bory. (Fulton, 1977), *Gonium pectorale* Müller (Sako *et al.*, 1991), *Gambierdiscus toxicus* Adachi and Fukuyo (Chinain *et al.*, 1997) and various other freshwater and marine dinoflagellates (e.g. Hayhome *et al.*, 1987; Sako *et al.*, 1989). Also, in the present study, morphotypes of a particular species do not cluster

together. For example, within *Botrydiopsis callosa* (Fig. 68), morphotypes B6, B7, B8 and B9 are scattered over the dendrogram.

Clusters which are formed according to banding pattern similarities often contain strains of more than one morphotype and strains of a particular morphotype occur in separate clusters. For example, in *Botrydiopsis* (Fig. 68), strains of *B. callosa* B6 are in clusters A, C and D and strains of *B. alpina* B2 are in C and D. Also, group C clusters strains of seven morphotypes. Similarly, for *B. callosa* B7, one New Zealand strain is in cluster B and one Antarctic strain is in cluster C with other morphotypes. In *Chlorellidium* (Fig. 69), strains of *C. tetrabotrys* C1 and *C.* sp. A C2 are in both clusters A and B. In contrast, for *B. callosa* B8 a single strain from each of New Zealand and Antarctica are in cluster C. However, more strains from other origins are needed to confirm that all strains of *B. callosa* would cluster here.

No correlation is apparent between isozyme banding patterns and the geographic origin of isolates either in *Botrydiopsis* (Fig. 68) or *Chlorellidium* (Fig. 69). For example, in *B. alpina* B2, one Antarctic strain is in cluster D and two Antarctic strains are in cluster C together with the three European strains. Also, within *B callosa* B6, the seven Antarctic strains are in three separate clusters. For *C. tetrabotrys* C1, one Antarctic strain is in cluster A and four Antarctic strains are in cluster B together with the one European strain.

Strains of a particular species isolated from different localities within a region are in separate clusters. For example, two strains of *B. callosa,* G19/2 isolated from Granite Harbour and G99/2 from Castle Rock, are in different clusters.

Strains of a particular morphotype isolated from different samples taken at the same location are usually found in different clusters. For example, within *B. callosa* B6 (Fig. 68), five strains isolated from five samples taken at Granite Harbour, are widely scattered over the dendrogram. Also, two strains of *C. tetrabotrys* C1, G31/6 and G28/7, isolated from two samples from Granite Harbour, are in different clusters (Fig. 69).

In other studies, isozyme analysis grouped strains of *Alexandrium* Halim (formerly *Protogonyaulax*) (Cembella and Taylor, 1986) and *Peridinium volzii* Lemmerman (Hayhome *et al.,* 1987) according to geographical locations. Analysis of the moss *Sarconeurum glaciale* (C. Muell.) Card. and Bryhn, suggested that samples from Ross Island and southern Victoria Land form one population and those from the Vestfold Hills another, possibly as a result of separate colonisation events (Selkirk *et al.,* 1997). However, it is not possible here to define any clear geographic clusters of strains of *Botrydiopsis* and *Chlorellidium.*

In the present study, there is no evidence of clustering of strains based on morphotype, region and locality. However, six isolates of *Chlorellidium tetrabotrys* C1 from the same sample produced identical bands and form a distinct sub-cluster within group B (Fig. 69). It is possible that these isolates were derived from the same propagule which established a population throughout the sample material. Unfortunately, multiple isolates from the same sample of other morphotypes were not tested to detect variation at this level. Whether close clustering would occur in all cases is unknown.

Both *Botrydiopsis* and *Chlorellidium* have intraspecific variation as have *Thalassiosira* species which have been isolated from different localities or from the same locality at an interval of 10 y (Murphy and Guillard, 1976). Skov *et al.* (1997) also reported intraspecific variation in the diatom *Pseudonitzschia pseudodelicatissima* (Hasle) Hasle using isolates from the same locality. Medlin *et al.* (1995) noted genetic variability within single species is common in unicellular algae.

Genetic variation of *Botrydiopsis* and *Chlorellidium* has not been studied previously. The frequency of polymorphic loci, which indicates the genetic variability in a population, is 0.29 for *Botrydiopsis* and 0.43 for *Chlorellidium.* In comparison,

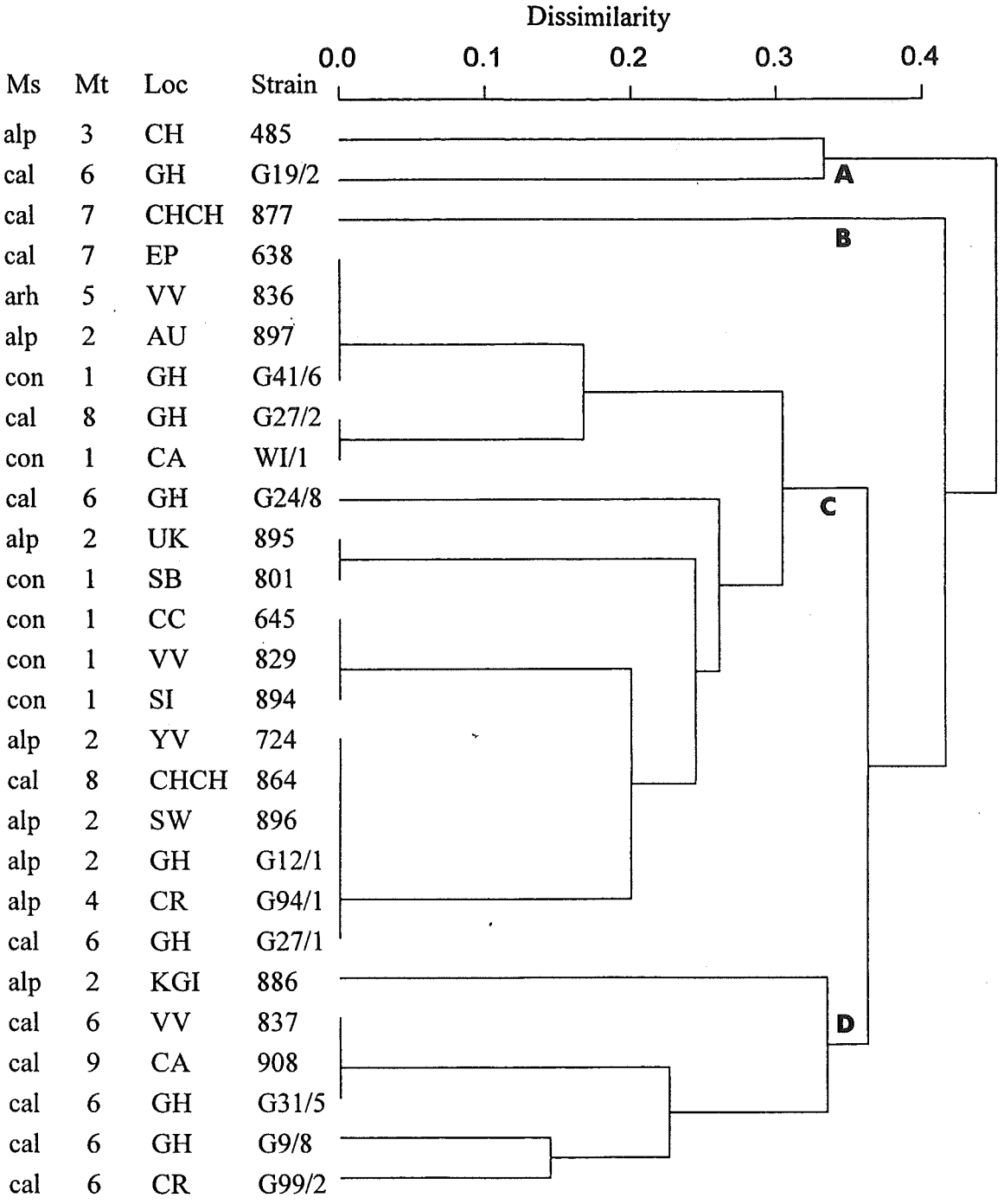


Fig. 68. Cluster analysis dendrogram using similarity coefficient with average linkage based on isozyme patterns of strains of nine morphotypes (Mt) of *Botrydiopsis* assigned to four morphospecies (Ms), *B. alpina* (alp), *B. arhiza* (arh), *B. callosa* (cal) and *B. constricta* (con) which have been recorded from different localities (Loc). For locality abbreviations see Table 2.1.

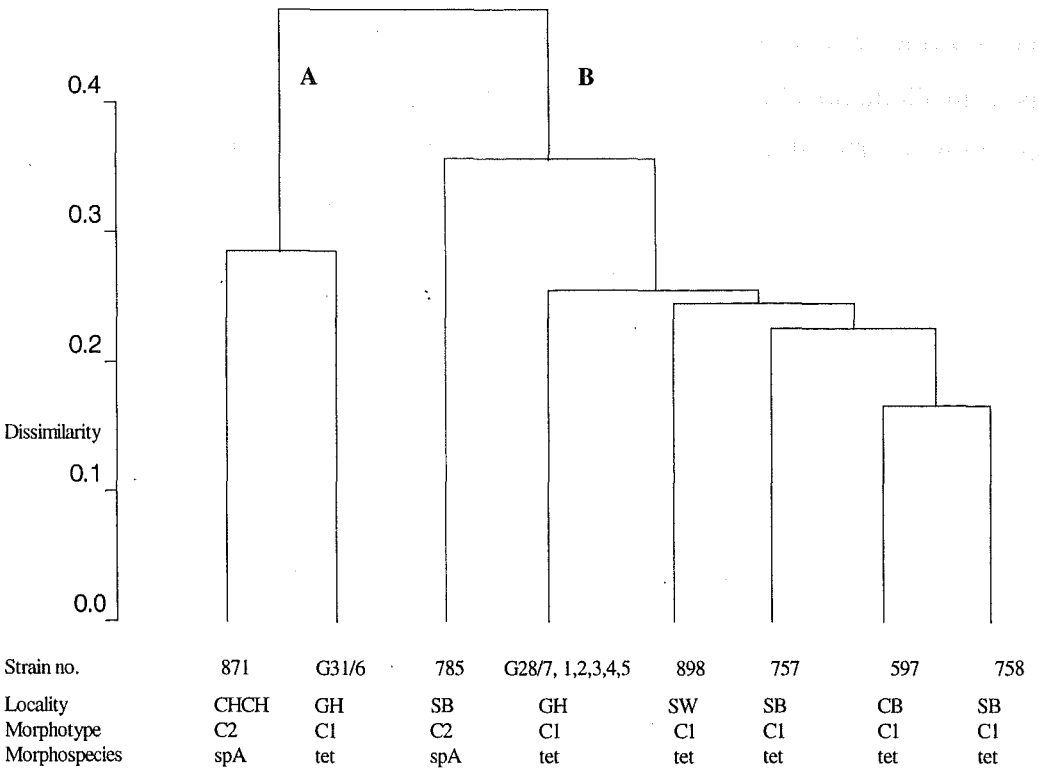


Fig. 69. Cluster analysis dendrogram using similarity coefficient with average linkage based on isozyme patterns of strains two morphotypes of *Chlorellidium* (C1-C2) assigned to two morphospecies, *C. tetrabotrys* (tet) and *C.* sp. A (spA) which have been recorded from different localities. Six isolates (1-5, G28/7) of the same morphotype from the same sample are cluster together. For locality abbreviations see Table 2.1.

frequencies of 0.2 to 0.6 have been reported for animals and vascular plants (Cheney and Babbel, 1978; Otrosina *et al.,* 1992).

Few isozyme studies have used microalgae, partly because of difficulties in defining allelles and loci. In vascular plants this can be accomplished by back crosses. As a consequence, population studies of such organisms must be based on interpretation of putative loci and alleles.

Bucheim *et al.* (1990) emphasized the several problems relating to genetic interpretation and data analysis of isozyme banding patterns derived from haploid cells. Heterozygotes cannot be produced by haploid cells, thus, multiple bands seen in a particular isozyme system, such as PGI and G6PDH, may represent protein variants or products from allelic/nonallelic genes. Genetic analyses are required to differentiate these possibilities. Therefore, it is difficult or impossible to analyze the present electrophoretic data in terms of gene frequencies (Swofford and Berlocher, 1987).

Murphy (1978) discovered that freshly isolated clones of the marine diatoms, *Thalassiosira pseudonana* Hasle and Heimdal and *Skeletonema costatum* (Grev.) Cleve., lose their heterozygosity within the first six months of culture due to frequent self-fertilization and auxospore formation. This raises questions about the relevance and significance of electrophoretic analyses for taxonomy. More knowledge is required of the stability of enzyme loci and the effects of environmental conditions before the usefulness of this approach can be assessed (Cox, 1993).

4.2.3 Comparison of phenetic and traditional approaches

Genetic variability within a species can be indicated by morphological variation amongst strains (Medlin *et al.*, 1991). However, during the present study, only minor morphological variation was found between strains of each of morphotypes, B1, B2, B6-B8, C1 and C2, in spite of considerable intraspecific genetic variation revealed by isozyme analysis. Further investigation is needed to confirm whether this variation is significant. Gallagher (1980) reported genetic variation of morphologically identical isolates of *Skeletonema costatum* using isozyme electrophoresis.

Costas (1990) and López-Rhodas and Costas (1997) suggest that natural populations of asexually reproducing cyanobacteria may accumulate genetic diversity by mutations and genetic drift. When genes which control morphology are affected, the different individuals could be interpreted as different morphospecies. However, individuals of identical morphology could be genetically different if those genes unrelated to morphology had been affected, e.g. cell surface components. This hypothesis can be applied to *Botrydiopsis* and *Chlorellidium* in which reproduction is only by asexual

spores and vegetative division. For example, *Botrydiopsis callosa* (B7) and *Chlorellidium* sp. A (C2) each have two morphologically identical strains, but isozyme analysis shows these to be genetically different.

In this study, congruency is lacking between the three methods used to group strains of *Botrydiopsis* and *Chlorellidium*, i.e. by phenetic analysis of morphological and isozyme data and by the traditional approach, based on unequal weighting of characters. Bucheim *et al.* (1990) came to similar conclusions in a study of *Sphaeroplea.* Therefore, further studies, such as 18S rRNA gene sequencing (e.g. Buchheim *et al.*, 1990; Goff and Coleman, 1988), are needed to test the congruency of different species concepts.

In the traditional approach, certain characteristics are given much greater weight than others. For example, presence or absence of vegetative division is the most important character for distinguishing *Chlorellidium* and *Botrydiopsis.* Also, the occurrence of specimens with characteristics intermediate between two species is not uncommon and when this occurs it is difficult to confidently place them in either. For example, the only characteristic which separates *B. alpina* and *B. constricta* is the frequency of cell division by constriction of adult cells. If information on this is lacking then specimens could be placed in either species as both are without a pyrenoid and both have a smooth cell wall and a stigma and 1 to 2 chloroplasts in the zoospore.

Phenetic analysis is useful in that it allows a more objective evaluation. There may therefore be more validity in using a synthetic classification which is based on overall similarity, especially considering that many classifications have been influenced to a large extent by the preconceptions of the researcher. Numerical taxonomy may resolve some problems, although the subjectivity involved in determining which characters and character states are to be used still remains.

4.2.4 Pigment analysis

There has been considerable interest in the pigment composition of the Xanthophyceae, especially the examination of their carotenoid pigments and its application to chemotaxonomy. Presence or absence of certain xanthophylls is valuable as a taxonomic marker for assigning algae to classes, but within classes seems of little utility unless a substantial number of species are examined (Rowan, 1989). Several attempts have been made to identify chloroplast pigments of the Xanthophyceae (e.g. Thomas and Goodwin, 1965; Whittle and Casselton, 1969; Sullivan *et al.*, 1990). Whittle and Casselton (1975a) commented on early investigations of pigment composition which gave erroneous identification of some xanthophylls. For instance, diadinoxanthin in *Tribonema aequale* (Whittle and Casselton, 1969; Thomas and Goodwin, 1965) and *Botrydiopsis alpina* (Thomas and Goodwin, 1965) were misidentified as antheraxanthin.

High performance liquid chromatography (HPLC) has become essential in pigment analyses because of its short analysis time, sensitivity, reliability and quantitative capabilities. In the present study, it has been used to investigate the application of pigments as chemotaxonomic biomarkers in *Botrydiopsis* and *Chlorellidium.*

As previous investigations (e.g. Stransky and Hager, 1970; Whittle, 1976) employing thin-layer chromatography indicated that not all species of Xanthophyceae contain chlorophyll *c,* Sullivan *et al.* (1990) suggested that its apparent absence could be due to undetectably low concentrations. The present finding supports the view that chlorophyll *c* is characteristic of the Xanthophyceae (Sullivan *et al.,* 1990; Whittle and Casselton, 1969) as it was present in all strains at concentrations which might not have been detectable using other chromatographic methods.

Strain *et al.* (1970) and Heukelem *et al.* (1992) emphasised the difficulty of identifying closely related xanthophylls such as diadinoxanthin and diatoxanthin, zeaxanthin and lutein, and α-carotene and β-carotene. These closely eluting pigments can be resolved by HPLC. However, HPLC techniques typically used in analysis of complex pigment mixtures are generally unable to separate chlorophyll *c1* and *c2* (e.g. Wright *et al.,* 1991; Kohata *et al.,* 1991) as found in the present study. In the present study, diadinoxanthin

has been detected in significant amounts which is in agreement with Whittle and Casselton (1975b).

Stransky and Hager (1970) used thin-layer chromatography to study the carotenoids of eight species of Xanthophyceae including the type culture of *Botrydiopsis alpina*. The same carotenoids as found in the present study were reported except for the presence of cryptoxanthin monepoxide and the absence of chlorophyll *c* and zeaxanthin.

In this study, the presence of chlorophyll *c* and zeaxanthin and the absence of cryptoxanthin was shown in the type culture of *B. alpina* as well as in 21 of 27 strains of *Botrydiopsis* and 7 of 8 strains of *Chlorellidium*. Whittle and Casselton (1975b) found forms of cryptoxanthin in *Tribonema aequale*, but here it was absent in both *Botrydiopsis* and *Chlorellidium*. Egger *et al.* (1969) reported zeaxanthin and diadinoxanthin in *Botrydium*, but Stransky and Hager (1970) claimed that *Botrydium* and other yellow-green algae which contain diadinoxanthin also contain diatoxanthin but never zeaxanthin. Heukelem *et al.* (1992) emphasised the difficulty of resolving the closely related pigments zeaxanthin, lutein and alloxanthin.

Violaxanthin was not detected. This confirms that none of the algae studied are members of the Eustigmatophyceae as violaxanthin is characteristic of that class (Hibberd and Leedale, 1971, 1972; Whittle and Casselton, 1975a). Vaucheriaxanthin or its derivatives is the characteristic xanthophyll for Xanthophyceae (Potter *et al.*, 1997) and was found in all strains. It is surprising that Strain *et al.* (1970) did not find these in *Tribonema*.

Trace amounts of three fucoxanthin-like pigments were detected in most strains of *Botrydiopsis* and *Chlorellidium*. Such pigments have only rarely been found in Xanthophyceae (Potter *et al.*, 1997).

Thirty-four carotenoids remained unidentified. Three were present at higher percentages (~8%) than other minor carotenoids (all <0.5%, Tables. 3.10, 3.11). Unknown

carotenoids have also been reported by Stransky and Hager (1970) in *Tribonema aequale* Pascher.

The pigment concentration of microalgae is very flexible to enable them to adapt to fluctuating light quality and intensity (Jeffrey *et al.,* 1997). The relative proportions of pigments can also change under different light regimes (Vesk and Jeffrey, 1977; Stransky and Hager, 1970). For instance, diadinoxanthin is transformed into diatoxanthin in light in Xanthophyceae and this is reversible in the dark. This corresponds to the violaxanthin to zeaxanthin transformation in higher plants. Different culture media can also cause qualitative changes, i.e. presence or absence of a particular pigments in algae (Jeffrey and Wright, 1994). Controlled growth conditions and a standard method of analysis must be used to achieve comparable results. There are no such published records for *Botrydiopsis* (Table. 4.5) and *Chlorellidium.*

Table. 4.5. Published records of pigment composition of *Botrydiopsis.*

Taxa	[a]Culture no.	Method used	Culture medium	Literature
Botrydiopsis alpina	SAG 806-1	[b]Thin-layer chromatography (TLC)	Modified BBM	Stransky and Hager,1970
B. alpina	CCAP	Column chromatography	Modified BBM	Thomas and Goodwin, 1965
B. cf. arhiza	M43	[c]Spectrophotofluorometry and TLC	BBM	Sullivan *et al.* 1990
B. cf. intercedens	M1	[c]Spectrophotofluorometry and TLC	BBM	Sullivan *et al.* 1990
B. sp.	M485	[c]Spectrophotofluorometry and TLC	BBM	Sullivan *et al.* 1990

[a] SAG, culture from Göttingen; CCAP, from Culture Collection of Algae and Protozoa, UK, strain number was not mentioned; M, from Melbourne.

[b] thin-layer chromatography inconjuction with infrared spectroscopy and microchemical tests for identifying functional groups of carotenoids.

[c] spectrophotofluorometry was used for detection of chlorophyll *c* and thin-layer chromatography for distinguishing major carotenoids, i.e. β-carotene, diadinoxanthin, diatoxanthin, heteroxanthin and vaucherioxanthin ester.

The carotenoid composition of *Botrydiopsis* and *Chlorellidium* is close to other xanthophyceans. In addition, there are no major qualitative and quantitative differences in pigment composition amongst the strains examined. Pigment content is not a useful diagnostic character at the generic or species level. This is in contrast to the

considerable diversity found amongst species of *Chlorococcum* (McLean, 1968) and the cyanoprokaryotes *Cyanobium, Synechococcus* and *Cyanothece* (Komárek, 1996).

4.3 Chlorophyta - comparison of Antarctic morphotypes with known species using the traditional taxonomic approach

During the present investigation, 73 strains have been assigned to 32 species of 14 genera (Table 4.6). Among them, 23 species and two genera are new records for Antarctica. No sufficiently detailed descriptions of morphological and reproductive features with life-cycle information are available for Antarctic specimens of any of these genera. Most identifications in the literature are left at generic level. For example, Broady (1986) tentatively assigned a coccoid unicell to *Neospongiococcum* without description and there are numerous other examples (as outlined in the introduction).

Table. 4.6. List of species of terrestrial chlorophyte algae described in the present study.

Species	Strain (s)	New records
Chlamydophyceae		
Chlamydomonadales		
Chlamydomonas cf. intermedia	766	
Unidentified species of *Chlamydomonas*	751, G99/4	A
Chlorococcales		
Chlorococcum cf. elkhartiense	633, 765	A
C. cf. infusionum	775	R
C. tatrense	821	A
C. sp. A sp. nov.	823	A
Neospongiococcum gelatinosum forma	761	A
Radiosphaera sp. A sp. nov.	833	A
Tetracystis sp. A sp. nov.	G5/8, 763, MB37/3	A
T. sp. A forma	G8/3, 647, 798, MB49/3	A
Macrochloris cohaerens forma	809, G9/4, G8/7	A
Chlorophyceae		
Chlorellales		

cont.

Table 4.5 (cont.)

Myrmecia macronucleata	FB45D	A
Chlorella ellipsoidea	752	
C. emersonii	637	
C. sp. A sp. nov.	635, G27/4, G9/1	A
C. cf. luteoviridis	826	A
C. vulgaris	G8/6, G28/3, G31/1, 639, 828, MB49/6	
Elliptochloris reisiglii nov. comb.	G41/2	A
E. reniformis	G19/5, MB38/3, MB42/3, ISO15, TH101RRL, FB102RR	
Bracteacoccus aerius	G12/6	A
Gloeotilales		
Binuclearia tectorum forma	762	
Chaetophorales		
cf. Protoderma	771	A
Ulvophyceae		
Pleurastrales		
Trebouxia corticola forma	839	A
T. crenulata	G95/7, G97/7, MB42/2	A
Charophyceae		
Klebsormidiales		
Stichococcus allas forma	*600, G19/3	A
S. bacillaris forma A	*MB18/4	A
S. bacillaris forma B	*MB7A/1	A
S. exiguus forma A	*G97/6	
S. exiguus forma B	*756, MB1/5	A
S. minutus forma A	*ISO19, ISO20, FB100RR/L, TH48D, JC104RR/L, JC49D	A
S. minutus forma B	*599, 631, G19/8, G41/1, MB1/1, MB18/2, MB37/7, MB64/1, MB42/5, MB63/2, MB40/4	A
cf. Stichococcus	*MB40/8	A

A, first record for Antarctica; R, first record for Ross Sea regions; all other taxa have been previously recorded elsewhere in Antarctica (Appendix 1); * strains were examined by TEM.

During the present study I have come across many discrepancies in the literature which I have discussed in the following sections. This suggests that probably the specimens were not properly identified because of lack of understanding of taxonomic characters of the species concerned.

Chlamydomonas

Chlamydomonas is one of the largest genera comprising more than 500 species (Ettl, 1976, 1983). Ettl and Schlösser (1992) revised the systematics of this genus based on sporangium wall autolysin, morphology, reproduction, development and physiological properties. Seventeen species have been identified from Antarctica (Appendix 1). In addition, five taxa are tentatively assigned to a species. 21 records simply state *Chlamydomonas* sp. and little confidence can be had in these generic identifications in many cases. Among 17 Antarctic *Chlamydomonas* species, only *C. chlorostellata* Flint and Ettl has been well described from Signy Island (Broady, 1979a). This has been included as a synonym within *C. meslinii* Bourrelly (Ettl and Gärtner, 1995). Remaining species are simply listed or inadequately described and/or illustrated. *C. antarctica* Wille in Gain, from Antarctic Peninsula (Gain, 1911a), Ongul Island (Akiyama, 1968) and Haswell Island (Kol, 1973), is regarded by Ettl (1976) as an uncertain species as it is insufficiently described.

In a comparison of 18S rRNA partial gene sequence of fourteen species of *Chlamydomonas,* five strains of *C. reinhardtii* Dang. were identical to each other but distinct from the other thirteen species (Buchheim *et al.,* 1990). In this case, the morphological species and the molecular species concepts agree, and moreover, a biochemical analysis of autolysin groups agrees as well (Schlösser, 1984). However, Buchheim *et al.* (1990) also included the genera *Volvox* Linn., *Haematococcus* Agardh and *Chlorogonium* Ehren. in the same study, and these genera are intermingled among the *Chlamydomonas* species, leading the authors to conclude that *Chlamydomonas* is a polyphyletic genus.

Earlier taxonomists relied solely on the morphology and cytology of the vegetative cell, ignoring important features of reproduction and development (Ettl and Schlösser, 1992).

When diagnosis was based on cultured cells only one clone was used in most cases. Therefore, the limits of species were too narrowly selected and do not conform to those of natural populations. Different species which are difficult to distinguish from one another are clustered together. A species diagnosis should therefore include, in addition to the vegetative phase, a characterization of morphological as well as physiological aspects of reproduction. A careful examination of spore formation and discharge, of gametogenesis, gamete fusion, formation, development and germination of zygotes and akinetes is necessary.

In the present study, two morphotypes of *Chlamydomonas* have been studied. Among them, one is assigned as *C. cf. intermedia* but another morphotype cannot be identified to species level. Further study is needed for distinguishing its features.

C. cf. intermedia (pages 156, 158-161)

Morphotype 1. This is close to *C. intermedia* except in that alga the cells are larger (15-20 μm by 8-14 μm) and an apical papilla is absent. However, Seaburg *et al.* (1979) identified Antarctic specimens from southern Victoria Land as *C. intermedia* despite their lack of an apical papilla. Because of these differences this morphotype is assigned as *C. cf. intermedia.*

Unidentified species of Chlamydomonas (pages 157-161)

Morphotype 2. This morphotype cannot be identified to species level using the keys of Ettl and Gärtner (1995) and Ettl (1976). It is close to *C. typica* Deason and Bold in chloroplast shape. However, *C. typica* has a median to posterior stigma, anterior nucleus, 2-4 spores in a sporangium and a pyrenoid with an entire starch sheath whilst morphotype 2 has an anterior stigma, median to posterior nucleus and 2-8 spores per sporangium. *C. typica* has not been recorded from Antarctica. This morphotype requires further study of morphology, reproduction and development.

Chlorococcum

Archibald and Bold (1970) and Ettl and Gärtner (1995) selected specific morphological traits such as: the nature of the starch sheath around the pyrenoid, shape of the chloroplast, cell size, thickness of the cell wall, the occurrence or not of akinetes with sculptured walls and contractile vacuoles in adult cells for characterizing species of *Chlorococcum*. 22 species are recorded from soil (Ettl and Gärtner, 1995) of which four (Appendix 1) have been recorded from Antarctica. Sixteen Antarctic records do not identify species. As little or no description of specimens is provided there can be little confidence in even generic identifications.

In the present study, four morphotypes are assigned to four morphospecies, *C. cf. elkhartiense*, *C. cf. infusionum*, *C. tatrense* and *C.* sp. A sp. nov. Comparisons between these morphotypes are shown in Table. 4.7.

Table 4.7. Comparison between the four morphotypes of *Chlorococcum* described in this study.

Characteristics	Morphotype			
	1	2	3	4
Adult cell				
Diameter (μm)	5.5-27.5	8-15.5	7.5-25	5-17
[a]Starch sheath around pyrenoid	S	S	S	2-5 grains
Zoospore				
Position of stigma	anterior	anterior	anterior	median to posterior
Position of nucleus	posterior	posterior	posterior	anterior

[a] S, sheath entire, perforated and lobed.

C. cf. elkhartiense (pages 162, 164-167)

Morphotype 1. This is very similar to *C. elkhartiense* Archibald and Bold except for pyrenoid number and structure (Ettl and Gärtner, 1995). In *C. elkhartiense,* a single spherical pyrenoid is surrounded by a continuous starch sheath while one to two pyrenoids with entire, lobed and perforated starch sheaths are present in morphotype 1. This morphotype is assigned as *C. cf. elkhartiense. C. elkhartiense* has not been described from Antarctica previously.

Ettl and Gärtner (1995) include ten species with an entire starch sheath. In *C. elkhartiense,* the perforations in starch sheath have not been observed previously with LM, but only noted for other *Chlorococcum* species in an earlier TEM study (Brown and McLean, 1969). The spherical cell, lobed chloroplast and number (>16) of autospores place morphotype 1 closest to *C. elkhartiense.*

C. cf. infusionum (pages 162-165)

Morphotype 2. This is close to *C. infusionum* (Schrank) Meneghini (Ettl and Gärtner, 1995), but differs in the structure and number of pyrenoids and the more restricted size range of cells (Table. 4.8). These differences suggest that this morphotype can be assigned as *C. cf. infusionum.*

Table. 4.8. A comparison of morphotype 2 with *Chlorococcum infusionum.*

Characteristics	Morphotype 2	*C. infusionum*
Adult cell		
Diameter (μm)	8-15.5	10-40
Pyrenoid		
[a]Starch sheath	S	grains
Number	1	1-2

[a]S, sheath entire, perforated and lobed.

C. infusionum has been well described from Signy Island as *C. humicolum* (Broady, 1979a). Ettl and Gärtner (1995) transfered *C. humicolum* (Nägeli) Rabenhorst to *C. infusionum.* Morphotype 2 is identical to Signy Island specimens.

C. tatrense (pages 163-167)

Morphotype 3. This is identical with *C. tatrense* (Ettl and Gärtner, 1995). This is the first record of this species for Antarctica.

Chlorococcum sp. A sp. nov. (pages 158-159, 163, 166-168)

Morphotype 4. This cannot be identified to species level using Ettl and Gärtner (1995) and Archibald and Bold (1970). Morphotype 4 is compared with other morphotypes in Table 4.7. In features of adult cells it appears close to *Chlorococcum* sp. isolated from the Antarctic Peninsula by Broady (1979b), but no description of zoospores is provided. This makes comparison difficult. It is also close to *C. pinguideum* Arce and Bold in shape of vegetative cells and chloroplast, and absence of contractile vacuoles, but differs as follows: vegetative cell is larger in *C. pinguideum* (up to 47 μm diameter), stigma in zoospores is anterior in *C. pinguideum* but median to posterior in morphotype 4. Because of these distinct differences, a new species, *Chlorococcum* sp. A is proposed.

> Diagnosis: "Cells spherical to ellipsoidal, 5-17 μm diameter, uninucleate. Cell wall smooth, thin. Chloroplast cup-shaped. Pyrenoid spherical to ellipsoidal, up to 3 μm diameter, mostly one occasionally two, embedded in the lateral thick region of the chloroplast, surrounded by 2-5 starch grains. Sporangia spherical to ellipsoidal, up to 12.5 μm diameter, containing 2 to 6 spores. Sporangium wall smooth, thin. Spores released by rupture of sporangium wall. Aplanospores spherical to ellipsoidal, 3-9 μm diameter. Zoospores ellipsoidal to fusiform, 5-10 by 3-8 μm, with either a small, flat or two hump-like apical papillae, flagella up to 10 μm long, two anterior contractile vacuoles, a single anterior nucleus, and a chloroplast with a lateral pyrenoid, and a median to posterior ellipsoidal stigma."
>
> Holotype: Figs. 42.23-33 and 45.8.

Locality: Isolated from soil, Victoria Valley ($77^0 23'$S, $161^0 25'$E), 1991.

Neospongiococcum

Neospongiococcum Deason (1971a) is morphologically very similar to *Spongiococcum* Deason. Unlike *Spongiococcum,* vegetative cell division is absent in *Neospongiococcum.* Ettl and Gärtner (1995) illustrated seven species of *Neospongiococcum* which have been found in soil. However, there has been no detailed description of this alga from Antarctica. The only Antarctic records are of specimens tentatively assigned to *Neospongiococcum* from the Vestfold Hills (Broady, 1986) and Scott Base (Broady and Smith, 1994). No descriptive details were given.

In the present study, morphotype 1 is assigned to *N. gelatinosum* forma.

N. gelatinosum forma (pages 168-173)

Morphotype 1. This is similar to *N. gelatinosum* (Archibald and Bold) Ettl and Gärtner except for the structure and number of pyrenoids, size of zoospores, and number of aplanospores. In *N. gelatinosum*, a single pyrenoid is surrounded by a continuous starch sheath, the zoospore is smaller (8 μm long) and eight to 16 aplanospores are formed. In contrast, morphotype 1 has mostly one to occasionally three pyrenoids, each of which is covered with an entire, lobed and perforated starch sheath. The zoospore is slightly larger (up to12.5 μm long) and 2-16 aplanospores are formed. These differences are very minor and therefore this morphotype is regarded as *N. gelatinosum* forma.

Radiosphaera

There are two species, *R. minuta* Herndon and *R. negevensis* Ocampo-Paus and Friedmann, both of which have been recorded from soil (Ettl and Gärtner, 1995; Ocampo-Paus and Friedmann, 1966). Neither has been recorded from Antarctica. Starr (1955) provided a detailed description of *R. dissecta* (Korshikov) Starr based on an isolate from soil. This alga has been briefly described from southern Victoria Land

(Seaburg *et al.*, 1979) and listed from McMurdo Sound (Flint and Stout, 1960) but has been transferred to *Actinochloris sphaerica* Korshikov (Ettl and Gärtner, 1995). Ettl and Gärtner (1995) provides descriptions and illustrations of *R. minuta* and *R. negevensis*. Diagnostic features of species are as follows. In young cells, the chloroplast is H-shaped in *R. minuta* but asteroidal in *R. negevensis*. *R. minuta* has a small, indistinct papilla while in *R. negevensis*, two distinct, hump-like papillae are present.

Radiosphaera sp. A sp. nov. (pages 169-173)

Morphotype 1. This cannot be identified to species level using Ettl and Gärtner (1995) and Ocampo-Paus and Friedmann (1966). It is closest to *R. negevensis* Ocampo-Paus and Friedmann but in that species cells are smaller (to 16 μm diameter) and zoospores are larger (12-17 μm by 7-12 μm) than in morphotype 1. Also, the starch sheath is granular in *R. negevensis*. A new species, *Radiosphaera* sp. A, is proposed.

> Diagnosis: "Cells mostly solitary, occasionally forming loose aggregates, spherical to ellipsoidal, 10-36 μm diameter. Contractile vacuoles two, opposite to nucleus. Cell wall smooth, thin. Chloroplast axile, asteroidal, with forked lobes extending from central portion to cell wall and which flatten against wall and appear as polygonal plates in surface view. Pyrenoid spherical to ellipsoidal, 6 μm diameter, embedded in the central portion of chloroplast. Starch sheath entire, perforated and lobed. Sporangia spherical to ellipsoidal, up to 17.5 μm diameter, containing 2-32 spores, four aplanospores frequent. Sporangium wall smooth, thin, with mucilage sheath. Spores released by dissolution of sporangium wall. Aplanospores ellipsoidal to spherical, 5-13 μm diameter. Zoospores ellipsoidal, 8-13 by 6-8.5 μm, with two hump-like papillae, a single axile chloroplast with a central pyrenoid, median to anterior ellipsoidal stigma, two anterior contractile vacuoles, and a single median nucleus."

Holotype: Figs. 46.8-15 and 47.5-8.

Locality: Isolated from soil, Victoria Valley ($77^0 23'$S, $161^0 25'$E), 1991.

Tetracystis

Ettl and Gärtner (1995) compile descriptions of 17 *Tetracystis* species for which they have confidence. Diagnostic features are very similar to those used for *Chlorococcum.* There are nine records of *Tetracystis* sp. from Antarctica (Appendix 1). However, lack of descriptions and adequate illustrations prevent species assignments for these. In addition, *T. cf. intermedia* has been described from southern Victoria Land (Seaburg *et al.*, 1979). More descriptive features of morphology and reproduction are needed for this species to be assigned confidently to *T. intermedia.*

In the present study, a new species, *Tetracystis* sp. A, and a form of this are proposed.

***Tetracystis* sp. A sp. nov. (pages 174-179)**

Morphotype 1. This is close to *T. dissociata* Brown and Bold except for the pyrenoid which in morphotype 1 is surrounded by an entire, lobed and perforated starch sheath, while the starch sheath is granular in *T. dissociata.* Of the 17 species described by Ettl and Gärtner (1995) only *T. fissurata* Nakano possesses an entire starch sheath around the pyrenoid, but this is not perforated (Nakano 1984; Table 4.9). The perforations in the starch sheath could have been missed with LM and this needs re-examination. *T. fissurata* possess a chloroplast with deep and complex lobes which is significantly different from morphotype 1. Morphotype 1 is distinct enough to be described as a new species, *Tetracystis* sp. A.

> Diagnosis: "Cells in groups of two or four, many tetrads loosely associated by mucilage sheath but tetrad complexes not formed. Cells spherical to ellipsoidal, 8-18 μm diameter. Nucleus single situated in the lumen of chloroplast. Contractile vacuoles two. Cell wall smooth, thin. Mucilage sheath up to 2 μm in diameter. Chloroplast parietal, lobed, with fissures,

stripe-like in surface view. Pyrenoid spherical to ellipsoidal, up to 6 μm diameter. Starch sheath thick, entire, perforated and lobed. Carotenoid accumulation in old culture. Sporangia spherical to ellipsoidal, up to 22.5 μm diameter, containing two to eight spores. Spores released by rupture of sporangium wall. Sporangium wall smooth, thin. Aplanospores spherical to ellipsoidal, 5-15 μm diameter. Zoospores cylindrical to ellipsoidal, 3.5-10.5 by 3-8.5, with either flat or a hump-like papilla, two equal flagella about the same length as the cell, single parietal chloroplast with a lateral pyrenoid, median to anterior ellipsoidal stigma, two anterior contractile vacuoles, and a single posterior nucleus. Vegetative division forming diads and mostly tetrads of daughter cells closely associated with parental cell wall."

Holotype: Figs. 48.1-9 and 49.1-3.

Locality: Isolated from sandy soil, richly encrusted with the green alga *Prasiococcus calcarius*, Granite Harbour ($77^0 01'$S, $162^0 30'$E), 1996; from soil, Scott Base ($77^0 50'$S, $166^0 48'$E), 1991 and from gravel with green algal crust, Washington Ridge, Marie Byrd Land ($77^0 30'$S, $154^0 00'$W), 1996.

Table 4.9. A comparison of *Tetracystis fissurata* and *T. dissociata* with morphotypes 1 and 2.

Characteristics	*T. fissurata*	*T. dissociata*	Morphotype 1	Morphotype 2
Octahedral complex	not formed	formed	not formed	formed
Adult cell				
Size (diameter, μm)	13-20	14-18	8-18	9-23
Chloroplast	deep and complex lobed margin	cup-shaped, lobed margin	lobed margin	cup-shaped, lobed margin
Starch sheath around pyrenoid	E	G	S	S
Zoospore				
Length (μm)	7-9	8-10	3.5-10.5	5-10
Width (μm)	3-5	2.5-4	3-8.5	2.5-7
Shape	ellipsoidal	cylindrical	cylindrical to ellipsoidal	ellipsoidal to fusiform
Aplanosporangium complex	present	absent	absent	absent

E, entire, not perforated; G, granular; S, entire, perforated and lobed.

Tetracystis sp. A forma (pages 175-179)

Morphotype 2. Morphotype 2 differs slightly from morphotype 1 in the size of the vegetative cell and the formation of octahedral complexes (Table 4.9). Because of these minor differences it is assigned as *T. antarctica* forma.

Macrochloris

Macrochloris is close to *Actinochloris* Korshikov but differs in the shape of the chloroplast and the number of pyrenoids. *Macrochloris* has an axial, irregularly lobed chloroplast and 1-10 irregular pyrenoids which are surrounded by either a starch sheath or starch granules, whilst in *Actinochloris,* the chloroplast is axial, asteroidal and the pyrenoid is single and surrounded by starch granules. Of the five species, *M. cohaerens* (Vintzer) Ettl and Gärtner, *M. multinucleata* (Reisigl) Ettl and Gärtner and *M. chlorococcoides* Ettl and Gärtner are reported from soil (Ettl and Gärtner, 1995). Only *M. multinucleata* has been reported from Antarctica, at the Windmill Islands region, but without accompanying descriptions (Ling and Seppelt, 1998).

M. cohaerens forma (pages 180, 182-185)

Morphotype 1. This most closely resembles *M. cohaerens* in cell size, chloroplast shape and zoospore shape, but differs in the position of nuclei in vegetative cells. All nuclei are clustered in *M. cohaerens* while in morphotype 1, they are scattered as in *M. multinucleata.* This morphotype has smaller vegetative cells than *M. multinucleata* which attain 40-100 μm diameter. Also, the zoospore of *M. multinucleata* has two hump-like papillae while it is flat in morphotype 1. However, the zoospore of this morphotype is slightly longer than those of both *M. cohaerens* (10-12 μm by 4-4.5 μm) and *M. multinucleata* (10 μm by 4-5.5 μm). Morphotype 1 also differs from *M. dissecta* Korsch. and *M. radiosa.* Both the latter have an anterior nucleus in the zoospores and larger vegetative cells which attain 52 μm diameter. Also, a single contractile vacuole is present in the zoospore of *M. dissecta* whereas two are present in remaining species as well as morphotype 1. In *M. chlorococcoides*, the pyrenoid is surrounded by starch grains in which it differs from morphotype 1 and other species. Morphotype 1 has a

pyrenoid which is surrounded by an entire, perforated and lobed starch sheath and in other species it is entire and lobed but not perforated. This morphotype is most similar to *M. cohaerens* and differences are so minor that it can be assigned as *M. cohaerens* forma.

Myrmecia

Ettl and Gärtner (1995) describe seven species of *Myrmecia.* There are two Antarctic records, *M. bisecta* Reisigl and *M. irregularis* (J. B. Petersen) Ettl and Gärtner. Broady (1979a) provided a detailed description of *M. bisecta* from Signy Island where it was frequently recorded as a moss epiphyte and a soil alga. The description conforms quite closely to the original except that no mention is made of an eyespot in the chloroplast of the zoospore as shown by Ettl and Gärtner (1995, fig. 104d). This species was also recorded from Windmill Islands without accompanying descriptions (Ling and Seppelt, 1998). Another species, *M. irregularis* was recorded from Ongul Island (Akiyama, 1968) as *Bracteacoccus irregularis* J. B. Petersen. This record is accompanied by a poor illustration and the description is brief and without reference to reproductive stages. It can be regarded only as doubtful.

M. macronucleata (pages 181, 184-185, 188-189)

Morphotype 1. This can be assigned to *M. macronucleata* because of similar shape of the chloroplast and presence of a large, distinct nucleus. This is the first record of *M. macronucleata* from Antarctica.

M. bisecta differs from *M. macronucleata* by having larger cells, up to 35 μm diameter, a thin cell wall and an indistinct nucleus. *M. irregularis* differs from both *M. bisecta* and *M. macronucleata* by the irregular shape of the vegetative cell (Ettl and Gärtner, 1995).

Chlorella

The taxonomy of *Chlorella* is very difficult because of its morphological simplicity. However, major taxonomic studies have been based on morphology (Fott and Nováková, 1969; Komárek and Fott, 1983; Ettl and Gärtner, 1995). These recognise 14 species from terrestrial and aquatic habitats and as lichen phycobionts. Of the 12 terrestrial species, eight have been recorded from Antarctica. Three species recorded in the Antarctic literature, *C. conglomerata* (Artari) Oltmans, *C. kottlitzi* (Fritsch) Wille and *C. antarctica* (Fritsch) Wille have not been accepted as valid species. There are 46 Antarctic records of *Chlorella* species (Appendix 1), but only three are a result of critical comparison with species described in the modern literature. These identify *C. emersonii* (Broady, 1984b; Broady *et al.,* 1987), *C. saccharophila* (Broady, 1984b) and *C. protothecoides* (Broady, 1984b) from cultures. It is apparent that *Chlorella*-like algae are widespread, however, all records which have not used cultures are doubtful. Without the use of unialgal cultures full details of the life-cycle remain unknown and the necessary information on chloroplast shape, position and extent is difficult or impossible to obtain.

Recent molecular genetic studies (e.g. Booton *et al.,*1998) have indicated that *Chlorella* is a form genus grouping together genetically distant entities of similar morphology. For instance, *C. minutissima* Fott and Nováková and *C. kessleri* Fott and Nováková are clustered in a trebouxiophycean clade and are genetically distinct from *C. fusca* Shihira and Krauss which is within a chlorophycean clade.

In the present study, five morphotypes (Table. 4.10) assigned to five morphospecies, *C. vulgaris, C.* sp. A sp. nov., *C. cf. luteoviridis*, *C. emersonii* and *C. ellipsoidea* are discussed.

C. vulgaris (pages 186, 188-191)

Morphotype 1. This can be assigned to *C. vulgaris*. It is widely reported from Antarctica, but most records are either simply names in lists, e.g. from Vestfold Hills (Broady, 1986), Windmill Islands (Ling and Seppelt, 1998), Scott Base (Broady and

Smith, 1994) or are inadequately described and illustrated, e.g. from southern Victoria Land (Seaburg *et al.*, 1979) and cannot be regarded as reliable.

Table. 4.10. Comparison between five morphotypes of *Chlorella* described in this study.

Characteristics	Morphotype				
	1	2	3	4	5
Adult cell					
[a]Size (μm)	3.5-11 d	3-8.5 d	7.5-16 d	4-11.5 d	9-18 l x 6-14.5 w
[b]Chloroplast	C, G	Bl	C, B	C, P, L	C, B
Pyrenoid	+	-	+	+	+
Autospore					
[c]Size within an autosporangium	E	E	U	E	U
Number	2-16	2-16	2-16	2-100	2-32

[a]d, diameter; l, length; w, width.

[b]chloroplast shape: B, band-shaped with lobed margin; Bl, bi-lobed; C, cup-shaped; G, girdle-shaped; L, lobed; P, perforated.

[c]E, equal; U, one spore larger than others.

Chlorella sp. A sp. nov. (pages 186, 188-191)

Morphotype 2. In its lack of a pyrenoid this resembles *C. minutissima* and *C. homosphaera* Skuja, but differs in some other characteristics (Table. 4.11).

C. homosphaera as described by Broady (1979a) from Signy Island is up to 18 μm diameter and the chloroplast completely covers the wall, but these features were not observed in morphotype 2. This morphotype has a bi-lobed chloroplast which is

significantly different from other species. It is suggested that morphotype 2 should be erected as a new species, *Chlorella* sp. A.

Table 4.11. A comparison of morphotype 2 with other *Chlorella* species.

Characteristics	Morphotype 2	*C. homosphaera*	*C. minutissima*
Adult cell			
Size (diameter, µm)	3-8.5	5-7	1-3.5
Shape	spherical to ellipsoidal	spherical to ellipsoidal	spherical
Chloroplast	[a]bi-lobed	cup, band to mantel-shaped	band to cup-shaped
Autospore	2-16	2-16	2-8
[b]Autosporangium wall	P	P	N

[a]Chloroplast is bi-lobed with a connection between the two broad lobes.

[b]P, persists in the culture medium; N, dissolved in the culture medium.

Diagnosis: "Cells spherical to ellipsoidal, 3-8.5 µm diameter. Chloroplast bi-lobed, cup-shaped in side view, covering 3/4 of cell wall, lacking pyrenoid. Autosporangia spherical to ellipsoidal, up to 10.5 µm diameter, containing 2-16 autospores. Autospores released by irregular rupture of sporangium wall which persists in culture medium. Autospores mostly spherical, occasionally ellipsoidal, 2-4.5 µm diameter."

Holotype: Figs. 52.9-18 and 53.4-6.

Locality: Isolated from moss cushions encrusted with a thin black layer of cyanobacteria, Granite Harbour ($77^0 01'$S, $162^0 30'$E), 1996 and from soil, Inexpressible Island ($74^0 52'$S, $163^0 40'$E), 1985.

C. cf. luteoviridis (pages 187-191)

Morphotype 3. This is similar to *C. luteoviridis* Chodat, because of size and shape of cell, shape of chloroplast and unequal size of autospores. However, it differs slightly, as in *C. luteoviridis* the autosporangium contains 2-32 autospores which are released through a regular aperture. Because of these differences morphotype 3 is assigned as *C. cf. luteoviridis. C. luteoviridis* has not been described from Antarctica previously.

C. emersonii (pages 187-191)

Morphotype 4. This is identical to *C. emersonii* which is distinguished from other species by its perforated chloroplast. This morphotype has smaller cells than specimens from geothermal soil on Mt. Erebus, Ross Island (Broady, 1984b) and on Mt. Melbourne, northern Victoria Land (Broady *et al.*, 1987) which are 15 μm and 18 μm diameter respectively.

C. ellipsoidea (pages 188-192)

Morphotype 5. This is identical to *C. ellipsoidea* except that no more than 32 autospores were observed. The main distinguishing features of this alga are the shape of the chloroplast which is cup to band-shaped with an undulate margin, the greater number (2-64) and unequal size of autospores, and the pyrenoid which is surrounded by several starch grains.

In Antarctica, *C. ellipsoidea* is reported only from freshwater habitats of northern Victoria Land (Fumanti *et al.,* 1995), but without descriptions or illustrations. It does not give confidence in the identification and his specimens need detailed study in culture.

Elliptochloris

An important diagnostic feature of this genus is the production of autospores of two distinct shapes, ellipsoidal and spherical (Ettl and Gärtner, 1995). All three species, *E. bilobata* Tschermak-Woess, *E. reniformis* (Watanabe) Ettl and Gärtner and *E.*

subsphaerica (Reisigl) Ettl and Gärtner have been recorded from Antarctica. All these records (Appendix 1) can be regarded as reliable as they describe and/or illustrate this feature and are based on examination of cultures.

Recently, Hanagata *et al.* (1998) proposed *Watanabea* gen. nov. and *Watanabea reniformis* sp. nov. They proposed this genus based on the comparative study of cell wall structure *Elliptochloris bilobata* and two strains which is believed conspecific with *Chlorella reniformis* Watanabe. *E. bilobata* has two layers of cell wall with a trilaminar sheath and *C. reniformis* has one layer of cell wall.

Ettl and Gärtner (1995) transfered *Pseudochlorella subsphaerica* Reisigl to *Elliptochloris* and used it as the basionym. However, I do not agree with Ettl and Gärtner's recombination as *P. subsphaerica* does not produce autospores of two distinctly different shapes and cannot be placed in *Elliptochloris*. Reisigl described *P. subsphaerica* as having oval to reniform vegetative cells, whereas no description was made of the shape of autospores. Only a single autosporangium with eight ellipsoidal autospores was illustrated (Reisigl, 1964, his fig. 28, c). It seems that it produces just one type of autospore. However, further study of autospores is needed to confirm this. Ettl and Gärtner (1995) suggests that *Chlorella reisiglii* (Reisigl) Watanabe is synonymous with *E. subsphaerica. C. reisiglii* would be a more appropriate basionym because it produces two types of autospores and vegetative cells, each with a pyrenoid. Therefore, I suggest a new recombination, *E. reisiglii.*

E. reniformis (pages 192-193, 196-199)

Morphotype 1. This is identical to *E. reniformis*. The characteristic which distinguishes it from *E. reisiglii* is the absence of a pyrenoid.

Chlorella reniformis Watanabe was transfered to *E. reniformis* by Ettl and Gärtner (1995). It is recorded from Antarctica as *C. reniformis* from the Vestfold Hills (Broady, 1986) and northern Victoria Land (Broady *et al.,* 1987). Morphotype 1 is also identical to these specimens.

E. reisiglii nov. comb. (pages 193, 196-199)

Morphotype 2. This is identical to *E. reisiglii.* The vegetative cell of this morphotype is similar to *P. subsphaerica* (Reisigl, 1964), but the latter produces just one spore type. *E. reisiglii* has been recorded from Ross Island (Broady, 1984a), Vestfold Hills (Broady, 1986) and Scott Base (Broady and Smith, 1994) as *C. reisiglii.* Morphotype 2 is smaller than Ross Island specimens in which cells attain 12 μm diameter.

Bracteacoccus

Bracteacoccus consists of 15 species (Ettl and Gärtner, 1995). Only two, *B. minor* (Chodat) Petrová and *B. irregularis* (J.B. Petersen) Starr have been recorded from Antarctica. These records are poorly illustrated and the descriptions are brief and without reference to reproductive stages. This does not allow confidence in their identifications. The latter species, which is recorded from West Ongul Island (Akiyama, 1968), has been recombined as *Myrmecia irregularis* (J.B. Petersen) Ettl and Gärtner (Ettl and Gärtner, 1995). *B. minor* has been recorded from green snow on Balleny Island (Kol and Flint, 1968) and Terre Adélie (Kol, 1971), on geothermal soil on Ross Island (Broady, 1984b), as a sublithic alga at the Vestfold Hills (Broady, 1986) and in benthic mats in ponds at Schirmacher Oasis, Drønning Maud Land (Pankow *et al.*, 1991; Pankow and Haendel, 1995). It seems that this genus could be widespread in Antarctica, but further comparison of Antarctic isolates is required.

Lewis (1997) studied the diversity and phylogeny of *Bracteacoccus* with respect to other chlorophycean algae based on 18S ribosomal RNA gene sequence data. Nine strains representing five species, *B. aerius* Bischoff and Bold, *B. giganteus* Bischoff and Bold, *B. grandis* Bischoff and Bold and *B. medionucleatus* Bischoff and Bold from USA, *B. minor* (Chodat) Petrová from Europe, and three unidentified species (Broady 420, 668 and 686) from Antarctica and one (Watanabe HKN28) from Japan, grouped closely together. Her result supports the monophyly of *Bracteacoccus* strains.

B. aerius (pages 194, 196-199)

Morphotype 1. This is identical to *B. aerius*. The main distinguishing features of this alga are the shape of the zoospores and their possession of a small posterior stigma and thickening of adult cell walls with age (Ettl and Gärtner, 1995).
B. aerius has not been reported previously from Antarctica. None of the Antarctic strains, studied by Lewis (1997), were close to *B. aerius*.

Binuclearia

Binuclearia has four species of which details are known of three (Ramanathan, 1964). The species within the genus are not well characterised. There are two Antarctic records, *B. tatrana* Wittrock and *B. tectorum* (Kütz.) Beger and Wichmann. However, Wichmann (1937) suggests that *B. tatrana* is synonymous with *B. tectorum*. All 11 Antarctic records are based on field specimens and not cultures. Illustrations are provided by only Baker (1967) and Broady (1982b). *B. tectorum* differs from *B. eriensis* Tiffany and *B. zaisanica* Skvortzow by its size. *B. tectorum* is wider (up to 7.5 μm) than *B. eriensis* (up to 3 μm) but narrower than *B. zaisanica* (up to 36 μm).

B. tectorum forma (pages 194-197, 202-205)

Morphotype 1. This is very similar to *B. tectorum* except for its longer cells. Cells are up to 20 μm long in *B. tectorum.* Because of this minor difference morphotype 1 can be assigned as *B. tectorum* forma.

The alga is very similar to that described from Czechoslovakia by Lukavský (1970) except that the pyrenoid is more distinct and surrounded by larger starch granules in the Antarctic material. This alga is identical to specimens described from Taylor Valley, Antarctica (Broady, 1982b). There is no previous report on germination of akinetes. In a culture study, *B. tectorum* was considered to be distinct from the similar *Ulothrix mucosa* Thru. (Lokhorst and Vroman, 1974). It is recorded from Victoria Land (as *B. tatrana,* Baker, 1967), Drønning Maud Land (Pankow *et al.,* 1991) and from aquatic habitats in the Windmill Islands (Ling and Seppelt, 1998). It has also been reported

from Haswell Islands and Thalla Hills Oasis (Opalinski, 1972a, b) but without detail descriptions or illustrations and thus identification remains unreliable.

It is interesting to note that *Geminella mutabilis* (de Brébisson) Wille and the uniseriate stage of *Trichosarcina mucosa* (Broady) Chappell and O'Kelly resemble some forms of Antarctic field specimens identified as *B. tectorum* (Broady, 1982b). However, during the present study, cultures of *B. tectorum* did not produce either the characteristic multiseriate form of *T. mucosa* or the thick, extensive mucilage sheath of *G. mutabilis.* Therefore, *Binuclearia* appears to be a valid genus.

Protoderma

Protoderma is generally considered to contain two species, *P. sarcinoidea* (Groover and Bold) Tupa and *P. cohaerens* (Wittr.) Printz. Possession of a sarcinoid or pseudoparenchymatous thallus in *P. sarcinoidea* distinguishes it from *P. cohaerens* in which the thallus is irregular with isodiametric cells. The original diagnosis of *Protoderma* was extremely brief and no reproductive stages were mentioned. This was emended by Tupa (1974) to include algae with a prostrate thallus composed of irregularly arranged cells without erect branches and producing biflagellate zoospores each with a stigma and a pyrenoid. She transferred *Pleurastrum sarcinoideum* Groover and Bold to *Protoderma sarcinoidea* on the basis of the biflagellate nature of zoospores each with a visible pyrenoid, 2-8 zoospores per zoosporangium, lack of erect branching, and a thallus surrounded by a distinct mucilage sheath. Watanabe and Floyd (1992) demonstrated that zoospore ultrastructure of *P. sarcinoidea* resembles that of the ulotrichalean, *Gayralia oxysperma* (Kützing) Vinogr. and placed it in the Ulvophyceae. This is supported by 18S rRNA gene sequence data (Booton *et al.,* 1998).

Vegetative stages of *Protoderma* Kützing resemble those of *Pleurastrum* Chodat. Chodat (1894) described *Pleurastrum* as occurring as either single cells, or in tetrahedral groups of cells, or ultimately in a pleurococcoid state. The presence or absence of a stigma and of a pyrenoid in zoospores was not mentioned by Chodat (1894). The pleurococcoid stage which he illustrated consisted of subspherical to slightly elongate cells, each with a large parietal chloroplast and a pyrenoid, forming a primitive, shortly

branched filament. Ultrastructural information on zoospores of *Pleurastrum* is still lacking. However, a recent molecular genetic study by Lewis (1997) has indicated that *Pleurastrum* is a form genus grouping together genetically distinct entities of similar morphology. For example, she groups *P. insigne* Chodat with the chlorophyceans and this is genetically distinct from *P. terrestre* Fritsch and John which groups with the trebouxiophyceans.

There is obviously still considerable confusion regarding the concept of these two genera. Cultures of type specimens are not available for comparison and doubts concerning their generic characters are therefore difficult to resolve (Lukešová, 1991). Both genera are in need of revision. The solution is probably by further TEM study of zoospores and molecular genetic analysis.

Protoderma cohaerens has been recorded from the South Orkney Islands as *P. brownii* Fritsch (Fritsch, 1912b). There is no report of *Pleurastrum* from Antarctica.

cf. Protoderma (pages 200, 202-205)

Morphotype 1. According to the emended diagnosis of *Protoderma* (Tupa, 1974), this morphotype is close to *P. sarcinoidea* but differs in that *P. sarcinoidea* has a single pyrenoid (Ettl and Gärtner, 1995) and sporangia containing two to eight zoospores. Without further ultrastructural and molecular genetical studies, this morphotype cannot be confidently assigned to *Protoderma* and is therefore left as *cf. Protoderma.*

Trebouxia

According to Ettl and Gärtner (1995) *Trebouxia* comprises 27 species. For separating species, they used characteristics such as whether the cells are solitary or in tetrads, the shape of the chloroplast, cell size, the number of pyrenoids, the type of pyrenoid, and the presence or absence of a stigma in zoospores.

Tschermak-Woess (1989) described the development of autospores, aplanospores and zoospores in *Trebouxia.* She divided *Trebouxia* into two subgenera. Subgenus

Trebouxia was considered to reproduce by autospores, aplanospores and zoospores, whilst subgenus *Eleutherococcus* (Warén) Tschermak-Woess was considered to lack autospores and reproduce by zoospores and aplanospores. Friedl (1993) described two different cell-cycles in asexual reproduction and used these as taxonomic characters. In one cell-cycle, cell divisions after zoospore settlement result in autosporangia with 4-32 adhering autospores (tetrads or autospore packages). Each autospore then develops into either a zoosporangium or an autosporangium containing >32 small spores. In the other cell-cycle, however, the zoospores first develop into almost completely differentiated vegetative cells which are transformed directly into zoosporangia or autosporangia with >32 small spores. Autospore packages are not formed in this cell-cycle. Ettl and Gärtner (1984) noted that no clear delimitation of both types of sporangia containing non-motile cells is possible in *Trebouxia* since they only differ by the number of cells produced which is variable and therefore both are autosporangia. They did not use autosporulation as a taxonomic character. Friedl (1993) disagreed with Tschermak-Woess (1989) and Ettl and Gärtner (1984) and concluded that these differences in the reproduction by autospores are important characters for the identification of *Trebouxia* species, but do not justify separation of this genus into two subgenera.

Two species, *T. incrustata* and *T. cf. impressa* have been recorded from Antarctica as lichen photobionts (Aoki *et al.* 1998).

T. corticola forma (pages 201, 206-209)

Morphotype 1. This resembles *T. corticola* (Archibald) Gärtner, but the latter has smaller (up to 14 μm diameter) cells. Morphotype 1 is also similar to *T. arboricola* Puymaly and *T. anticipata* Ahmadjian and Archibald, but differs in some significant features (Table 4.12). This morphotype is assigned as *T. corticola* forma.

T. crenulata (pages 201, 206-210)

Morphotype 2. This is identical to *T. crenulata* (Table. 4.12). Its main distinguishing characteristics are the possession of a crenulated chloroplast with a single pyrenoid and production of up to 32 spores.

T. corticola differs from *T. crenulata* by having a lobed chloroplast with usually two, and occasionally up to seven, pyrenoids and up to 16 spores in each sporangium (Ettl and Gärtner, 1995).

Table 4.12. A comparison of morphotypes 1 and 2 with *Trebouxia crenulata, T. corticola, T. arboricola* and *T. anticipata.*

Characteristics	Morphotype 1	Morphotype 2	*T. crenulata*	*T. corticola*	*T. arboricola*	*T. anticipata*
Adult cell						
Diameter (µm)	10.5-21	6-22.5	10-20	8-14	13-15	9-18
Pyrenoid						
Shape	irregular	angular	angular	irregular	irregular	spherical
Number	1-7	1	1	2-4	1	1
Autospores						
Number	2-16	~32	~32	4-16	2-16	~64
Zoospore						
Size						
length (µm)	4-7.5	4-7	7-9	5	6	5
width (µm)	3.5-6	3-5	3-5	3	3	3
Stigma	+	+	+	+	-	+

+ present; - absent.

Stichococcus

Thirty species of *Stichococcus* have been described, however many of these are synonymous (Printz, 1964, Ettl and Gärtner, 1995). Eight have been recorded from soil, as air-borne propagules and as lichen photobionts (Ettl and Gärtner, 1995). Of these, only three species, *S. bacillaris*, S. *exiguus* Gerneck and *S. minutus* Grintzesco and Péterfi have been recorded from Antarctica.

Stichococcus is a very simple alga. Fragmentation following vegetative division, i.e. newly formed wall layers are continuous and contiguous with the existing parent wall, is the only method of reproduction reported. However, during the present study rare autosporulation was seen in a single morphotype. Eight morphotypes were recognised

based on LM characterictics and seven have been placed in four species (Tables 4.6; 4.13). The anomalous autospore forming morphotype is assigned as *cf. Stichococcus.* All have similar ultrastructural characteristics. Cells are equipped with a minimal set of organelles: a single nucleus, mitochondrion, chloroplast, peroxisome, golgi bodies and vacuoles.

Different studies have provided conflicting descriptions of pyrenoid features in *S. bacillaris*. Ettl and Gärtner (1995), using LM alone, described a naked pyrenoid in *S. bacillaris* while a TEM study by Pickett-Heaps (1975) located several starch grains around a pyrenoid in *S. chloranthus* (= *S. bacillaris*). In the present study, a naked pyrenoid is present in one morphotype and of variable presence in another morphotype of *S. bacillaris* (Table 4.13). It appears that possession of a pyrenoid is not an important character for delimiting species. A further study of the pyrenoid is necessary for a clear definition of this species.

Silverberg (1975) observed morphological changes involved in pyrenoid maturation of *Stichococcus* sp. In young cells, the pyrenoid consisted of a lenticular matrix of very weakly electron-opaque granular material with unusual splits which subdivided the pyrenoid. At later stages of development these were absent. During the present study, no such splits were seen. The structure of the pyrenoid as observed by Silverberg (1975) is similar to the description of Pickett-Heaps (1974) except for the presence of splits in the pyrenoid. However, both studies were based on different strains of *Stichococcus.*

S. minutus (pages 211, 216-219, 222-223)

Morphotype 1. This is closest to *S. minutus* because of the narrow cells and absence of a pyrenoid. This morphotype is assigned as *S. minutus* forma A.

Table 4.13. A comparison of seven *Stichococcus* morphotypes (1-7) described in the present study with *S. minutus, S. bacillaris, S.exiguus* and *S.allas* as described in Ettl and Gärtner (1995).

Characteristics	1	2	*S. minutus*	3	4	*S. bacillaris*	5	6	*S.exiguus*	7	*S.allas*
Filament											
[a]Cell number	4	5	4	15	9	2-10	4	10	8	14	2-8
Shape	I	I	I	I	I	I	I	I	I	S	S
Cell size (μm)											
Length	4-4.5	6-7.5	4-6	5.5-7	8.5-9.5	3.5-12	10-14	9-12.5	8-30	9-11	3.5-12
Width	2-2.5	2.5-3	2.5-3	3.5-4	3.5-4	2-3	3-3.5	3-3.5	1.8-2.5	3.5-4	1.5-3
Pyrenoid	-	-	-	±	+	+	-	-	-	-	-

[a] maximum number of cells; filament readily fragmented.

I, irregular; S, sigmoid; + present; - absent; ± Pyrenoid absent or naked where present

Morphotype 2. This is similar to *S. minutus* and morphotype 1 and is assigned as *S. minutus* forma B. Morphotype 2 develops cells which can be longer than in both *S. minutus* and morphotype 1.

S. minutus has been described from Signy Island (Broady, 1979a) but those specimens are shorter than both morphotypes.

S. bacillaris (pages 212, 216-219, 222-225)

Morphotype 3. This resembles *S. bacillaris* except for the variable presence of a pyrenoid and narrower range of cell length. Morphotype 3 is slightly wider than *S. bacillaris.* It is assigned as *S. bacillaris* forma A.

Morphotype 4. This is close to *S. bacillaris* and morphotype 3, but differs in consistent possession of a pyrenoid. Morphotype 4 is slightly wider than *S. bacillaris* and longer than morphotype 4. It is assigned as *S. bacillaris* forma B.

S. exiguus (pages 212-213, 216-219, 224-225)

Morphotype 5. This is close to *S. exiguus.* The main distinguishing features of *S. exiguus* are absence of a pyrenoid and long vegetative cells (Ettl and Gärtner, 1995). Morphotype 5 has a smaller range of cell length and is slightly wider than *S. exiguus.* It is assigned as *S. exiguus* forma A.

This is the first report on ultrastructure of *S. exiguus.* This alga is recorded from East Ongul Island, Antarctica (Akiyama, 1968) but without a detailed description.

Morphotype 6. This is similar to *S. exiguus* and morphotype 5. It differs from morphotype 5 by its greater number of cells in a filament (up to10). Morphotype 6 is slightly wider than *S. exiguus.* However, ultrastructural features are similar. It is assigned as *S exiguus* forma B.

S. allas Reisigl (pages 213, 216-219, 224-225)

Morphotype 7. This is similar to *S. allas* in the possession of sigmoid filaments which is the feature separating it from other species. The filaments of morphotype 7 contain up to 14 cells while filaments of *S. allas* have two to eight cells. Morphotype 7 is slightly wider than *S. allas.* Because of these minor differences this morphotype is assigned as *S. allas* forma.

cf. Stichococcus (pages 214, 216-221)

Morphotype 8. This resembles *S. bacillaris* in filament shape and length, cell shape and size, ultrastructure and vegetative division but differs from this and other *Stichococcus* species in the rare formation of autospores. Autosporulation is absent in *Stichococcus* according to the present concept of the genus. This morphotype is close to *Nannochloris* in vegetative division (Ettl and Gärtner, 1995) and *Choricystis* in autosporulation (Krienitz *et al.,* 1996) but neither of these have both modes of reproduction (Table 4.14).

Table 4.14. A comparison of morphotype 8 with *Stichococcus bacillaris, Nannochloris,* and *Choricystis.*

Characteristics	Morphotype 8	*Stichococcus bacillaris*	*Nannochloris*	*Choricystis*
Adult cell				
Shape	bacilliform	bacilliform	spherical to ellipsoidal	ellipsoidal
Size (µm)				
Length	5-6.5	3.5-12	3.5-12	1.5-6
Width	3-3.5	2-3	2.5-3	1-3
Pyrenoid	+	+	+	-
Mode of reproduction				
Vegetative division	+	+	+	-
Autosporulation	+	-	-	+
Number of autospores	2-9	-	-	2-4

+ present; - absent.

Cell division in *Stichococcus* has been studied thoroughly by Pickett-Heaps (1974, 1975). The spindle is open, persistent at telophase and cytokinesis is effected by a cleavage furrow. No phycoplast is associated with the cleavage. Based on cell-division, *Stichococcus* has been placed in the Klebsormidiophyceae (Hoek *et al.,* 1995). Information on mitosis and cytokinesis is still lacking in *Nannochloris* and *Choricystis.* The ultrastructural studies on morphotype 8 were inadequate for resolving the detail observed by Pickett-Heaps.

The placement of *Choricystis* in Chlorococcales, Chlorophyceae, is supported by 18S rRNA gene sequencing data (Krienitz *et al.,* 1996). According to the traditional system, *Nannochloris* is a member of the Chlorophyceae (Ettl and Gärtner, 1995), but a molecular genetic study is needed to confirm this.

Confident generic assignment of morphotype 8 must await more detailed ultrastructural examination and molecular genetic assessment. It is premature to either erect a new genus or to emend the generic diagnosis of *Stichococcus* to include autosporulation. Because of this uncertainty and the need for further work, this morphotype is tentatively assigned as cf. *Stichococcus.*

4.4 Conclusions

This section highlights the main findings of this study whilst placing them in a broader context. It points to remaining gaps in knowledge and makes suggestions to fill these.

The following conclusions can be drawn from this study:

- Traditional approach to classification

    - As 33 of the total 39 species identified are known from elsewhere, this supports the contention that the Antarctic flora is mostly cosmopolitan.

    - Six species comprising two from Xanthophyceae (*Botryochloris* sp. A and *Chlorellidium* sp. A) and four from Chlorophyta (*Chlorella* sp. A,

Chlorococcum sp. A, *Radiosphaera* sp. A and *Tetracystis* sp. A) are proposed as new to science.

- Twenty-two species are new records for Antarctica as a whole and one is new to Ross Sea regions in particular.

- More detailed descriptions of the vegetative and reproductive characteristics have been provided for taxa which had previously been identified from Antarctica by other researchers but simply listed or inadequately described.

- Rare autosporulation was seen by LM in a *Stichococcus*-like alga and was confirmed by TEM.

- Hot fixation for TEM was used for the first time on microscopic algae. It proved successful in several strains for revealing details of ultrastructure, especially pyrenoid structure of *Botrydiopsis.* The first reports are provided on ultrastructure of *Botryochloris* and *Chlorellidium.* The first record of a pyrenoid in *Chlorellidium* was confirmed by TEM. This strain is proposed as a new species, *Chlorellidium* sp. A. Also, thylakoid bands with or without interconnections, and variations in thylakoid arrangement, were found in *Botrydiopsis* and *Chlorellidium.* In xanthophyceans, no report has been made previously on the reduction in thylakoid numbers along short lengths of the girdle lamella as found in *Chlorellidium tetrabotrys.*

- Classification by phenetic analysis of morphological characteristics

  - There was no distinct clustering of strains by generic or species identity according to the traditional approach.

- Classification by phenetic analysis of isozyme banding patterns

  - Isozyme analysis was not suitable for investigation of relationships at either generic, species or infraspecies levels in *Botrydiopsis, Botryochloris* and

Chlorellidium. Isozyme patterns did not define either genera, or species of a particular genus or morphotypes of a particular species.

- There was no evidence of clustering of strains based on geographical origin. Isozyme analysis did not cluster strains of: i) a particular species according to region of origin, ii) a particular species according to locality of origin within a region, and iii) a particular morphotype isolated from spatially separated samples taken at the same locality.

- Clustering did occur where multiple strains of a particular morphotype were isolated from a single sample.

- Lack of congruency between traditional and phenetic taxonomic analyses

  - The phenetic analysis of morphological data was not congruent with isozyme data and results of both these analyses were not in agreement with the traditional system of classification. The latter is based on unequal weighting of characters. For example, in distinguishing species within *Botrydiopsis,* presence or absence of a pyrenoid and thickness of the cell wall in adult cells, together with presence or absence of a stigma and number of chloroplasts in zoospores, are given much greater weight than other features. However, in the phenetic analysis used here all morphological features were equally weighted. This resulted in an entirely different clustering of strains.

- Carotenoid analysis

  - Pigments are not suitable chemotaxonomic biomarkers in *Botrydiopsis* and *Chlorellidium* as carotenoid pigment composition was very similar amongst all strains of *Botrydiopsis* and *Chlorellidium.* Neither an individual genus nor particular species possessed a distinct pigment combination. Only minor qualitative and quantitative differences were found amongst strains. Thirty-four unidentified carotenoids were found in both genera but in low concentrations.

The following outlines the main directions which could usefully be taken by future research.

- Traditional approach to classification

    - All future new records of Antarctic non-marine algae should be accompanied by thorough descriptions and illustrations. The practice of publishing new records without these has created many problems. It is often impossible to have confidence in an identification if there are no illustrations in the form of either *camera lucida* drawings or photomicrographs. These can describe algae more effectively than words, and are an essential means of documentation.

    - Many of the genera listed in the checklist of Antarctic non-marine xanthophycean and chlorophyte algae (Appendix 1) are either morphologically simple, so that descriptions of even unrelated genera tend to overlap, or morphologically pleomorphic, so that their appearance changes in different environmental conditions. This makes identification difficult at even the generic level and most lists of species include a number of unidentified algae (e.g. Nienow and Friedmann, 1993). Nienow (1996) suggested that the only way to overcome this problem is to study each alga in culture, so that its life-history can be determined. This has been achieved in the present study. Similar approaches could be applied to a wide range of other species which have been recorded from Antarctica, e.g. species of the xanthophyceans *Chloridella, Ellipsoidion, Gloeobotrys, Monodus, Nephrodiella* and *Pleurochloris.*

    - The use of different fixation methods in TEM could be necessary for obtaining ultrastructural data. Hot fixation techniques might usefully be applied to taxa which respond poorly to standard approaches. For example, pyrenoid occurrence and structure, which are often indistinct in

xanthophyceans in LM, are important characteristics which are better observed by TEM.

- The possibility of unique taxa amongst Antarctic terrestrial algae is suggested by the observation of Friedmann *et al.* (1988) that many "appear to be new to science". This is supported by the six new species proposed in the present study. However, adequate searches are needed in other regions before it can be stated with some confidence whether these species are endemic to Antarctica.

- The observation of rare autosporulation in a *Stichococcus*-like alga suggests a broader investigation is warranted to determine whether or not it occurs in algae assigned to *Stichococcus* by previous workers. In morphology and reproduction the Antarctic *Stichococcus*-like alga is close to *Nannochloris* and *Choricystis*. These and other genera of chlorophyte algae with small cylindrical cells require close examination and possible taxonomic revision. Investigation of patterns of mitosis by TEM and molecular genetic studies would help establish the relationships of these algae.

- Isozyme analysis

  The clustering of strains of a single morphotype isolated from the same sample requires confirmation and its significance requires further investigation. In this study only one morphotype in a single sample was investigated. Occurrence of similar clustering could be simply tested by examining multiple isolates of other morphotypes from single samples. If the phenomenon is confirmed then a detailed examination of the spatial scale over which clustering is maintained would be valuable. This could indicate the degree of local dispersal from an initial single colonization event. Lack of clustering could indicate populations arising from either several colonization events or from genetic adaptations to different microhabitats. Similar hypotheses have been tested on Antarctic mosses (Melick *et al.*, 1994; Selkirk *et al.*, 1997).

Smith (1993) presented evidence for long-distance airborne dispersal of spores of exotic bryophytes to Maritime Antarctica and their retention of viability in the soil propagule bank. Presently we lack evidence for this process in terrestrial algae. Further analyses of genetic variation in isolates of the same morphospecies from Ross Sea regions and comparisons with isolates from other continental sites and temperate regions, should help clarify whether this flora originated from Antarctic refugia or from other continents.

- Comparison of traditional and phenetic taxonomic analyses

    - According to the traditional taxonomic approach, *Botrydiopsis, Botryochloris* and *Chlorellidium* are very similar in shape of vegetative cells and chloroplast characteristics. However, these genera are recognized, in spite of these similarities, on the basis of vegetative division with the formation of tetrads in *Chlorellidium* and on the presence of a distinct mucilage sheath in *Botryochloris.* The results of the phenetic approach with equal weighting of morphological and isozyme characters suggests that such single features are inadequate for distinguishing genera. Generic and species concepts in these algae require testing by powerful modern approaches of molecular genetics. So far, only *Botrydiopsis intercedens* has been included in trees based on SSU rDNA gene sequences (Andersen *et al.,* 1998).

- Carotenoid analysis

    - In the present study, thirty-four quantitatively minor carotenoids remain unidentified and detailed study by modern approaches is needed for their characterization. It is possible that some may be unique to xanthophycean algae.

Concluding remarks. The proportion of the Antarctic non-marine algal flora which is endemic to the region will not be known until sufficient studies have been made using a more reliable taxonomy. This has important implications for conservation. If Antarctica does contain unique species, then these may become extinct as a result of human disturbance and climate change. There is a need for better documentation of all taxa with detailed descriptions and good quality light and electron micrographs. The compilation of a taxonomically reliable and comprehensive flora would be a worthwhile goal. This could then contribute towards the rational management of human impact on the region by facilitating the acquisition of knowledge of habitat requirements and distribution patterns of individual, well-founded species.

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Appendix 1. Published records[a] of freshwater and terrestrial chlorophyte and xanthophycean algae from Antarctica[b] included in literature to 1998.

Species	Reference
Chlorophyta	
Chlamydophyceae	
Chlamydomonadales	
Chlamydomonadaceae	
Carteria alpina Schmidle	Luscinska and Kyc, 1993.
C. cordata Ettl	Luscinska and Kyc, 1993.
C. simplex Pascher	Akiyama, 1967, 1968.
**Chlamydomonas acuta* Korshikov in Pascher	Holm-Hansen, 1964; Baker, 1967; Seaburg *et al.*, 1979.
Chlamydomonas agloeformis Pascher	Akiyama, 1967, 1968.
C. antarcticus Wille	Gain, 1911a; Wille, 1924; Corte, 1966; Akiyama, 1968; Kol, 1972, 1973.
**C. ballenyana* Kol and Flint	Kol and Flint, 1968; Klaveness and Rueness, 1986; Wagner and Zaneveld, 1988; Pankow *et al.*, 1991;
C. ballenyana var. *minor* Kol	Kol, 1971.
C . basimaculata Pascher *et* Jahoda	Parker *et al.*, 1972.
C. caudata Wille	Fritsch, 1912b; Parker *et al.*, 1972.
C. chlorostellata Flint and Ettl	Broady, 1979a, 1979b.
**C. cf.cribrum* Ettl	Broady,1984a.
**C. cf. debaryana* Goroschankin	Broady, 1984a; Starmach, 1995.
**C. ehrenbergii* Goroschankin	Fritsch, 1912a, b; Wille, 1924; Seaburg *et al.*, 1979.
C. elliptica Korshikov	Parker *et al.*, 1972
C. globosa Snow	Seaburg *et al.*, 1979.
C. gracilis Snow	Parker *et al.*, 1972.
C. impressa (Pascher) Ettl *et* O.	Luscinska and Kyc, 1993.
C. cf. incerta Pascher	Luscinska and Kyc, 1993.
**C. intermedia* Chodat	West and West, 1911; Fritsch, 1912a, b; Thomas, 1965; Seaburg *et al.*, 1979.
C. intermedia forma antarctica West and West	Wille, 1924.
C. cf. lapponica Skuja	Luscinska and Kyc, 1993.
C. nivalis (Bau.) Wille	Wille, 1924; Corte, 1966; Curl and Becker, 1970; Longton and Holdgate, 1979; Saag, 1979; Pankow *et al.*, 1991.
C. pseudopulsatilla Gerloff	Ling, 1996; Ling and Seppelt, 1998.

[a] Nomenclature is that provided in the literature surveyed. No attempt has been made to critically assess taxa. Classification is according to Ettl and Gärtner (1995) and Komárek and Fott (1983).

[b] From Continental Antarctica and Maritime Antarctica (according to Smith, 1984b).

*Taxa recorded from Ross Sea region.

cont.

Appendix 1 (cont.)

**C. cf. snowiae* Printz [= *Chlamydomonas communis* Snow]	Parker *et al.*, 1972; Broady, 1984a, 1989c.
C. sphagnicola (Frtsch) Fritsch *et* Takeda	Ling and Seppelt, 1998.
**C.* Ehrenberg sp.	Fritsch, 1912b; Wille, 1924; Fukushima, 1959; Flint and Stout, 1960; Thomas, 1965; Akiyama, 1968, 1977, 1979; Parker *et al.*, 1972; Broady, 1979a, 1979b, 1986, 1987a, 1989c; Engelskjøn, 1981, 1986; Priddle and Belcher, 1982; Klaveness and Rueness, 1986; Luscinska and Kyc, 1993; Broady and Smith, 1994; Ling, 1996.
**C. subcaudata* Wille	West and West, 1911; Fritsch, 1912; Wille, 1924; Seaburg *et al.*, 1979; Wharton *et al.*, 1981, 1983; Broady, 1987a, 1989c; Pankow *et al.*, 1991; Ling and Seppelt, 1998.
**Chloromonas alpina* Wille	Fritsch, 1912, 1912a; Wille, 1924; Seaburg *et al.*, 1979.
C. bolyaiana (Kol) Gerloff *et* Ettl in Ettl	Saag, 1979; Pankow *et al.*, 1991.
C. brevispina (Fritsch) Hoham, Roemer and Mullet	Ling, 1996; Ling and Seppelt, 1998.
C. palmelloides Broady	Broady, 1979a; 1979b.
C. pichinchae Lagerheim	Ishikawa *et al.*, 1986.
C. polyptera (*Scotiella polyptera* Fritsch)	Ling, 1996; Ling and Seppelt, 1998.
C. rosae Ettl [syn.: *Chlamydomonas rosae* Ettl]	Broady, 1979a.
C. rubroleosa Ling and Seppelt [Syn.: *Chlamydomonas nivalis* (Bauer) Wille]	Ling and Seppelt, 1993, 1998; Ling, 1996.
Chloromonas sp. 1	Ling, 1996; Ling and Seppelt, 1998.
**Furcilia lobosa* Stokes	Seaburg *et al.*, 1979.
Polytoma Ehrenberg sp.	Parker *et al.*, 1972.
Pyramichlamys semiglobosa (Pascher) Ettl *et* O.	Luscinska and Kyc, 1993.
Volvocales	
Volvocaceae	
**Brachiomonas cf. submarina* Boh.	Broady, 1989c.
**B. submarina* forma *obtusa* Hazen in Pascher	Seaburg *et al.*, 1979.
Gonium sociale (Dujardin) Warming	Nozaki and Ohtani, 1992; Ling and Seppelt, 1998.
G. Müller sp.	Parker *et al.*, 1972.
Pandorina morum (Müller) Bory	Pankow *et al.*, 1991.
Pteromonas nivalis (Shuttlew) Chodat	Fritsch, 1912b.
Pteromonas sp.	Parker *et al.*, 1972.
Sphaerellopsis sp.	Fumanti *et al.*, 1993.

cont.

Appendix 1 (cont.)

Polyblepharidaceae	
**Polytomella* Aragao sp.	Seaburg *et al.*, 1979
Quadrichloris carterioides (Pascher *et* Jahoda) Fott	Luscinska and Kyc, 1993.
Phacotaceae	
**Thorakomonas feldmanii* Bourrelly	Seaburg *et al.*, 1979.
Tetrasporales	
Palmellopsidaceae	
Chlamydocapsa lobata Broady	Broady, 1977a, 1979a, 1979b.
C. Fott sp.	Broady, 1979a, 1979b.
Palmella sp. 1	Ling and Seppelt, 1998.
cf. Palmella texensis Groover and Bold (= *Palmellopsis texensis* (Groover and Bold) Ettl and Gärtner	Broady, 1986.
Palmellopsis gelatinosa Korshikov	Parker *et al.*, 1972; Starmach, 1995.
Palmellopsis Korshikov sp.	Ling, 1996; Ling and Seppelt, 1998.
Chlorococcales	
Chlorococcaceae	
Chlorococcum ellipsoideum Deason and Bold [syn.: *Hypnomonas ellipsoidea* Korshikov, *Chlorococcum ellipsoideum* (Korshikov) Philipose, *C. oviforme* Archibald, *C. perplexum* Archibald and Bold, *C. arenosum* Archibald and Bold, *C. pulchrum* Archibald and Bold]	Broady, 1979a.
**C. humicolum* (Nägeli) Rabenh.	Holm-Hansen, 1964; Cameron and Benoit, 1970; Broady, 1979a.
**C. infusionum* (Schrank) Menegh. [syn.: *Chlorococcum humicola* (Nägeli) Rabenhorst p.p. *sensu auct.*, *C. chlorococcoides* (Korshikov) Philipose, *Hypnomonas chlorococcoides* Korshikov, *C. infusionum* var. *macrostigmaticum* Moewus, *Tetracystis chlorococcoides* Watanabe]	Vialow and Sdobnikova, 1961; Holm-Hansen, 1964; Parker *et al.*, 1972.
C. infusionum forma *antarctica* Wille	Wille, 1924.

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Appendix 1 (cont.)

**C.* Meneghini sp.	Flint and Stout, 1960; Holm-Hansen, 1964; Akiyama, 1968; Fukushima, 1968; Cameron *et al.*, 1970; Parker *et al.*, 1972; Seaburg *et al.*, 1979; Broady, 1979b, 1984a, 1986, 1987a; Wharton *et al.*, 1983; Broady and Smith, 1994; Starmach, 1995.
Hypnomonas ellipsoidea Korshikov [= *Chlorococcum ellipsoideum*]	Starmach, 1995.
H. lobata Korshikov [syn.: *Chlorococcum lobatum* (Korshikov) Fritsch *et* John]	Broady, 1979a.
H. tuberculata Korshikov	Starmach, 1995.
**Neospongiococcum* Deason [syn.: *Spongiococcum* Deason p.p.*sensu* Deason]	Broady, 1986, 1987a; Broady and Smith, 1994.
Spongiococcum cf. multinucleatum Deason and Bold [= *Deasonia multinucleata* (Deason and Bold) Ettl and Komárek]	Luscinska and Kyc, 1993.
**cf. Spongiococcum* Deason sp.	Broady, 1982b, 1984a
**Tetracystis* Brown and Bold sp.	Wharton *et al.*, 1981, 1983; Broady, 1984a, 1986, 1987a; Broady and Smith, 1994; Fumanti *et al.*, 1995.
**T.* sp. (? *intermedium* Brown and Bold)	Seaburg *et al.*, 1979.
T. sp. 1	Ling and Seppelt, 1998.
T. sp. 2	Ling and Seppelt, 1998.
Unidentified genus of the order (?) Chlorococcales	Broady, 1979a.
*Unidentified green unicell	Gain, 1911a; Smith, 1984c; Broady, 1987a; Ohtani and Kanda, 1987; Broady *et al.*, 1987; Ryan *et al.*, 1989.
Actinochloridaceae	
Apodochloris irregularis Ling and Seppelt	Ling and Seppelt, 1998
Macrochloris multinucleata (Reisigl) Ettl and Gärtner	Ling and Seppelt, 1998.
**Radiosphaera dissecta* (Korshikov) Starr [= *Actinochloris sphaerica* Korshikov]	Flint and Stout, 1960; Seaburg *et al.*, 1979.
**cf. Radiosphaera* Pascher sp.	Flint and Stout, 1960; Broady and Smith, 1994.
Chlorophyceae	
Chlorellales	
Myrmeciaceae	
**cf. Dictyochloropsis* Geitler *em.*Tschermak-Woess	Broady, 1989a.

cont.

Appendix 1 (cont.)

D. splendida Geitler	Starmach, 1995.
? *Lobococcus* Reisigl sp. (= *Myrmecia* Printz)	Ling and Seppelt, 1998.
Myrmecia bisecta Reisigl	Broady, 1979a, 1979b; Ling and Seppelt, 1998.
M. irregularis (J. B. Petersen) Ettl and Gärtner [syn. *Bracteacoccus irregularis* (J. B. Petersen) Starr]	Akiyama, 1968.
Palmellaceae	
Borodinella polytetras Miller	Starmach, 1995
Hormotila blennista Trainor *et* Hilton	Pankow *et al.*, 1991.
cf. Hormotila Borzi sp.	Broady, 1986.
Hormotila Borzi sp	Ling and Seppelt, 1998.
Sphaerocystis bilobata Broady [= *Coenochloris bilobata* (Broady) Hindák]	Broady, 1976, 1979a.
**S. (Coenochloris?) oleifera* Broady [syn. *Coenocystis oleifera* (Broady) Hindák]	Broady, 1976, 1979a, 1979b; Broady *et al.*, 1976, 1987a.
S. oleifera var. *antarctica* Broady	Broady, 1982c, 1984b.
Sphaerocystis schroeteri Chodat	Parker *et al.*, 1972.
S. schroeteri forma *nivalis* Fritsch	Fritsch, 1912b; Corte, 1966; Hirano, 1979.
S. schroeteri var. *nivalis* Fritsch	Kol, 1973; Oguni *et al.*, 1989.
S. signiensis Broady [= *Coenochloris signiensis* (Broady) Hindák]	Broady, 1979a.
Unidentified genus of the family Hormotilaceae	Broady, 1979a.
Chlorochytriaceae	
Kentrosphaera antarctica Kol	Kol, 1968b.
**K. ?bristolae* Smith	Akiyama, 1967, 1968; Seaburg *et al.*, 1979; Broady, 1986.
K. bristolae Smith	Ling and Seppelt, 1998.
K. sp.	Holm- Hansen, 1964.
K. facciolae Borzi	Pankow *et al.*, 1987, 1991; Fumanti *et al.*, 1995, 1997.
Characiaceae	
Characium naegelii A. Br.	Akiyama, 1967, 1968.
cf. C. pringsheimii A. Br.	Priddle and Belcher, 1982.
Chloroplana terricola Hollerbach	Starmach, 1995.
Fernandinella alpina Chodat	Kol, 1968b; Ling and Seppelt, 1998.
Rhopalocystis oleifera Schussnig [= *Coleochlamys oleifera* (Schussnig) Fott]	Broady, 1979a.

cont.

Appendix 1 (cont.)

Chlorellaceae

Ankistrodesmus antarcticus Kol *et* Flint	Kol and Flint, 1968; Pankow *et al.*, 1991.
A. gracilis (Rein.) Korshikov	Starmach, 1995
A. setigerus (Schroed.) West	Parker *et al.*, 1972.
A. sp	Corte, 1966.
Chlorella antarctica (Fritsch) Wille	Wille, 1924; Kol, 1973.
C. conglomerata (Artari) Oltmanns [syn.: *Pleurococcus conglomeratus* Artari]	Wille, 1924.
C. ellipsoidea Gerneck	Fumanti *et al.*, 1995.
C. ellipsoidea forma *antarctica* Wille	Gain, 1911a; Kol, 1968b.
**C. emersonii* Shih. and Krauss	Broady, 1984b; Broady *et al.*, 1987.
C. homosphaera Skuja	Broady, 1979a.
C. koettlitzi (Fritsch) Wille [syn.: *Pleurococcus koettlitzi* Fritsch]	Wille, 1924.
**C. miniata* (Näg.) Oltmanns [syn.: *Palmellococcus miniatus* Chodat]	Holm-Hansen, 1964; Baker, 1967.
C. minutissima Fott and Nováková [syn.: *Palmellococcus homosphaera* (Skuja) Handa and Nakano, *Mychonastes homosphaera* (Skuja) Kalina and Puncochárová]	Fumanti *et al.*, 1993.
**C. protothecoides* Krüger	Broady, 1984a, 1984b; Broady and Smith, 1994.
**C. saccharophila* (Krüger) Migula [syn.: *Palmellococcus saccharophilus* (Krüger) Chodat]	Broady, 1984a, 1984b; Broady *et al.*, 1994; Starmach, 1995.
**C. saccharophila* var. *ellipsoidea* (Gern.) Fott and Nováková	Broady, 1984a, 1984b.
**C.* Beijerinck sp.	Flint and Stout, 1960; Holm-Hansen, 1964; Corte, 1966; Akiyama, 1967, 1968, 1977, 1979; Parker *et al.*, 1972; Broady, 1979b, 1981, 1982a, 1989; Ohtani and Kanda, 1987; Ryan *et al.*, 1989; Broady and Smith, 1994.
C. vulgaris Beijerinck [syn.: *Chlorella terricola* Hollerbach]	Vialow and Sdobnikova, 1961; Holm-Hansen, 1964; Cameron and Benoit, 1970; Parker *et al.*, 1972; Seaburg *et al.*, 1979; Broady 1979a, 1986; Nagashima *et al.*, 1992; Broady and Smith, 1994; Ling and Seppelt, 1998.
C. vulgaris var. *autotrophica* (Shihira *et* Krauss) Fott *et* Nováková	Broady, 1979a, 1979b.
Elliptochloris bilobata Tschermak-Woess	Aoki *et al.*, 1998.

cont.

Appendix 1 (cont.)

**E.* reniformis (Watanabe) Ettl and Gärtner [basio. C. *cf. reniformis* Watanabe; *Lobosphaera reniformis* (Watanabe) Komárek *et* Fott]	Broady, 1986; Broady *et al.*, 1987; Massalski *et al.*,1994.
**E. subsphaerica* (Reisigl) Ettl and Gärtner [basio. *Pseudochlorella subsphaerica* Reisigl; syn. *C. reisiglii* Watanabe]	Broady, 1979a, 1984a, 1986; Broady and Smith, 1994; Ling and Seppelt, 1998.
E. subsphaerica var. *antarctica* [basio. *Pseudochlorella subsphaerica* var. *antarctica* Broady]	Broady, 1982c.
Hemichloris antarctica Tschermak-Woess *et* Friedmann	Tschermak-Woess and Friedmann, 1984; Meyer *et al.*, 1988.
Kirchneriella obesa (W. West) Schmidle	Pankow *et al.*, 1987,1991.
Lobosphaera tirolensis Reisigl	Ling and Seppelt, 1998.
Monoraphidium braunii (Nägeli) Kom.-Legn. [= *Chlorolobion braunii* (Nägeli) Kom.]	Broady, 1979a; Starmach, 1995.
M. contortum (Thur.) Komarkova-Legnerova	Ling and Seppelt, 1998.
M. griffithii (Berkel.) Komarkova-Legnerova	Starmach, 1995.
M. komarkovae Nyg.	Pankow *et al.*, 1987, 1991.
M. pusillum (Printz) Komarkova-Legnerova	Starmach, 1995.
M. sp.	Ling, 1996; Ling and Seppelt, 1998.
cf. Muriella J. B. Petersen sp.	Broady, 1979b.
M. terrestris J. B. Petersen	Luscinska and Kyc, 1993.
M. terrestris var. *reticulata* Broady	Broady, 1982c, 1986.
Muriellopsis sphaerica Broady	Broady, 1982c, 1986.
Planktosphaerella terrestris Reisigl	Broady, 1979a.
**Pseudococcomyxa simplex* (Mainx) Fott [syn.: *Pseudococcomyxa adhaerens* Korshikov]	Broady, 1979a, 1982c, 1984a, 1986, 1987b, 1989a; Broady *et al.*, 1987; Broady and Smith, 1994; Ling and Seppelt, 1998.
P. Korshikov sp.	Broady and Smith, 1994.
Scotiella antarctica Fritsch [syn.: *Pteromonas willei* Gain]	Fritsch, 1912b; Wille, 1924; Corte, 1962, 1966; Parker *et al.*, 1972; Kol, 1973; Broady, 1979a.
S. nivalis (Shuttlew) Fritsch	Curl and Becker, 1970; Kol, 1971, 1972, 1973; Longton and Holdgate, 1979.
S. polyptera Fritsch	Fritsch, 1912b; Wille, 1924; Kol, 1971; Akiyama, 1977.
S. Fritsch sp.	Parker *et al.*, 1972; Priddle and Belcher, 1982.
**Scotiellopsis terrestris* (Reisigl) Puncochárová and Kalina	Broady, 1984a.
Tetraedron minimum (Braun) Hansgirg	Priddle and Belcher, 1982; Pankow *et al.*, 1987, 1991.

cont.

Appendix 1 (cont.)

Hydrodictyaceae	
cf. Pediastrum boryanum (Turp.) Menegh.	Priddle and Belcher, 1982.
P. duplex Meyen	Pankow *et al.*, 1987, 1991.
Signiosphaera multinucleata Broady [= *Pseudodictyochloris multinucleata* (Broady) Ettl and Gärtner]	Broady, 1977a, 1979a, 1979b.
Micractiniaceae	
**Golenkinia minutissima* Iyengar and Balakrishnan [= *Golenkiniopsis parvula* (Voronich.) Korshikov]	Seaburg *et al.*, 1979.
Botryococcaceae (Syn.: Dictyosphaeriaceae Bourrelly)	
Dictyosphaerium chlorelloides (Naum.) Komárek and Perman [syn.: *Dictyosphaerium minutum* J. B. Petersen]	Broady, 1982c, 1986.
D. dichotomum Ling and Seppelt	Ling and Seppelt, 1998.
D. elegans Bachmann	Akiyama, 1967, 1968.
D. minutum J. B. Petersen [= *D. chlorelloides* (Naumann) Komárek and Perman]	Broady, 1979a.
D. subsolitarium Van Goor	Pankow *et al.*, 1991.
Unidentified genus of the family Dictyosphaeriaceae	Broady, 1979a.
Radiococcaceae	
Coccomyxa confluens (Kützing) Fott	Pankow *et al.*, 1987, 1991.
**C. curvata* Broady	Broady, 1982c, 1984a, 1986.
**C. dispar* var. *antarctica* Kol	Kol, 1973; Holm-Hansen, 1964.
**C. gloeobotrydiformis* Reisigl	Broady, 1982c, 1984a,1986; Broady *et al.*, 1987.
C. lacustris (Chod.) Pascher in Jaag.	Starmach, 1995.
Coenochloris sp.	Ling and Seppelt, 1998.
cf. Coenocystis sp.	Broady, 1989a.
? Diogenes bacillaris (West) Bourrelly	Parker *et al.*, 1972.
Gloeocystis polydermatica (Kützing) Hindák	Ling and Seppelt, 1998.
**Gloeocystis* Nägeli sp.	West and West, 1911; Wille, 1924; Broady, 1984a.

cont.

Appendix 1 (cont.)

**G. vesiculosa* Nägeli	Seaburg *et al.*, 1979; Ling and Seppelt, 1998.
?Mycacanthococcus antarcticus Wille	Gain, 1911a; Wille, 1924; Corte 1966.
?M. cellaris forma antarctica Wille	Gain, 1911a; Wille, 1924; Corte, 1960, 1966.
?M. ovalis Gain	Gain, 1911a; Wille, 1924.
Palmodictyon varium (Nägeli) Lemmermann	Parker *et al.*, 1972.
P. viride Kütz. in Prescott	Hirano, 1979; Pankow *et al.*, 1991.
**Palmogloea protuberans* (Smith) Kützing [= *Gloeocystis polydermatica* (Kützing) Hindák]	Holm-Hansen, 1964.
Pseudotetraspora gainii Wille	Gain, 1911a; Wille, 1924; Fumanti *et al.*, 1995.
Schizochlamydella minutissima Broady	Broady, 1982c, 1986.
Oocystaceae	
Chondrosphaera cf. lapponica Skuja	Broady, 1979a.
Cryocystis brevispina [syn. *Chodatella brevispina* Fritsch]	Fritsch, 1912b; Corte, 1962, 1966; Akiyama, 1977, 1979.
C. brevispina forma *groenlandica* Kol [syn. *Chodatella brevispina* forma *groenlandica* (Kol) Kol]	Kol, 1971.
C. granulosa Kol [*Chodatella granulosa* Kol]	Curl and Becker, 1970; Kol, 1971.
Eremosphaera viride De Bary	Fritsch, 1912b.
Excentrosphaera viridis Moore	Starmach, 1995.
Oocystis borgei Snow	Parker *et al.*, 1972.
O. lacustris Chodat	Fritsch, 1912b; Corte, 1966; Parker *et al.*, 1972.
O. lacustris forma *nivalis* Fritsch	Corte, 1962.
O. minuta Guillard, Bold and Mac Entee	Broady, 1987a.
O. minuta var. *ellipsoidea* Broady	Broady, 1982c, 1984b, 1986.
O. solitaria Wittr. in Wittrock *et* Nordstedt	Fritsch, 1912b.
Oocystis Braun sp.	Parker *et al.*, 1972; Priddle and Belcher, 1982; Smith, 1984c.
O. submarina Lagerheim	Pankow *et al.*, 1991.
Trochiscia aciculifera (Lagerh.) Hansgirg	Pankow *et al.*, 1987, 1991; Starmach, 1995.
T. antarctica Fritsch	Fritsch, 1912b; Corte, 1962, 1966.
T. aspera (Reinsch) Hansgirg	West and West, 1911; Wille, 1924.
T. crassa Hansgirg	Fritsch, 1912; Wille, 1924.
T. granulata (Reinsch) Hansgirg	Parker *et al.*, 1972; Luscinska and Kyc, 1993.
T. hystrix (Reinsch) Hansgirg	Gain, 1911b; Fritsch, 1912b; Wille, 1924.
T. nivalis Lagerheim	Fritsch, 1912b; Wildeman, 1935.
T. reticularis (Reinsch) Hansgrig	Fritsch, 1912b; Pankow *et al.*, 1991.
T. rubra Kol	Saag, 1979; Pankow *et al.*, 1991; Luscinska and Kyc, 1993;
T. Kützing sp.	Parker *et al.*, 1972; Broady, 1979a.

cont.

Appendix 1 (cont.)

T. tuberculifera Gain	Gain, 1911c; Wille, 1924.
T. zachariasi Lemmermann	Parker *et al.*, 1972.
Coelastraceae	
Coelastrum asteroideum De Notaris	Pankow *et al.*, 1987, 1991.
C. microporum Nägeli	Fritsch, 1912b.
C. microporum forma *irregulare* Fritsch	Fritsch, 1912b.
C. morus West and West	Pankow *et al.*, 1987, 1991.
C. sphaericum Nägeli	Fritsch, 1912b.
Scenedesmaceae	
Crucigenia tetrapedia (Kirch.) West *et* West	Pankow *et al.*, 1987, 1991.
Crucigeniella apiculata (Lemm.) Komárek [syn. *Crucigenia apiculata* (Lemm.) Komárek]	Pankow *et al.*, 1991.
C. rectangularis (Nägeli) Komárek	Pankow *et al.*, 1987, 1991.
Scenedesmus acuminatus (Lagerheim in Wittrock) Chodat	Pankow *et al.*, 1991.
S. acutus Meyen	Pankow *et al.*, 1987. 1991.
S. armatus Chodat	Komárek and Ruzicka, 1966; Pankow *et al.*, 1991.
cf. S. bijuga (Turp.) Lagerh.	Priddle and Belcher, 1982.
S. bijugatus ß alternans (Reinsch) Hansg.	Kol, 1968b.
cf. S. obliquus (Turp.) Kützing	Priddle and Belcher, 1982; Kol, 1968b.
S. opoliensis Richter	Pankow *et al.*, 1987, 1991.
S. quadricauda (Turp.) Brébisson *sensu* Chodat [syn.: *Scenedesmus communis* Hegewald]	Pankow *et al.*, 1987, 1991.
Westella botryoides (West) de Wildeman	Parker *et al.*, 1972.
**Westella* Wildemann sp. (Syn.: *Tetracoccus* West)	Seaburg *et al.*, 1979.
Neochloridaceae	
**Bracteacoccus cf. minor* (Chod.) Petrova	Broady, 1984a, 1986.
B. minor var. glacialis Flint	Kol and Flint, 1968; Kol, 1971; Saag, 1979; Pankow *et al.*, 1991.
**B* Tereg sp.	Holm-Hansen, 1964; Seaburg *et al.*, 1979; Broady, 1979a, 1984a.
**Neochloris aquatica* Starr	Cameron, 1972a.
Neochloris Starr sp.	Parker *et al.*, 1972.
cf. Spongiochloris Starr sp.	Parker *et al.*, 1972; Broady, 1979b; Luscinska and Kyc, 1993.

cont

Appendix 1 (cont.)

Chlorosarcinaceae

**Chlorosarcina consociata* (Klebs) Smith	Holm-Hansen, 1964.
Chlorosarcina sp.	Ling, 1996.
**Chlorosarcinopsis* Gerneck sp.	Seaburg *et al.*, 1979; Broady, 1979a, 1979b; Broady and Smith, 1994.
Chlorosphaera antarcticus Fritsch	Fritsch, 1912b; Hirano, 1959; Corte, 1966; Fogg, 1967; Akiyama, 1967, 1968; Kol, 1972, 1973.
Desmotetra Deason sp. 1	Ling, 1996; Ling and Seppelt, 1998.
Desmotetra Deason sp. 2	Ling, 1996; Ling and Seppelt, 1998.
**cf. Planophila* Gerneck sp.	Broady, 1979a, 1984a; Broady and Smith, 1994.
*Unidentified Chlorosarcinalean	Broady, 1987a.

Gloeotilales nom. prov.
Gloeotilaceae nom. prov.

Binuclearia tatrana Wittr. in Smith	Hirano, 1959; Baker, 1967a, Parker *et al.*,1972; Opalinski, 1972a, b.
**B. tectorum* (Kützing) Beger in Wichmann [basio.: *Gloeotila tectorum* Kützing; syn.: *Gongrosira subsimplex* Rabenhorst, *Binuclearia tatrana* Wittrock]	Broady, 1982b, 1987a; Pankow *et al.*, 1987, 1991; Fumanti *et al.*, 1995.1997.
**B* Wittrock sp.	Holm-Hansen, 1964; Broady and Smith, 1994.
Elakatothrix parvula (Archer) Hindák [syn.: *Spirotaenia parvula* Archer]	Broady, 1979a.
Fottea pyrenoidosa Broady	Broady, 1979a.
F. stichococcoides Hindák	Izaguirre and Pizarro, 1998.
**Geminella mutabilis* (de Brébisson) Wille	Seaburg *et al.*, 1979.
G. Turpin sp.	Broady and Smith, 1994.
G. terricola J. B. Petersen	Broady, 1982c; Ling and Seppelt, 1998.
Gloeotila contorta Chodat	Kol, 1968b.
Hormidiopsis crenulata (Kützing) Heering	Hirano, 1972; Pankow *et al.*, 1987, 1991.
Hormidium mucosum J. B. Petersen	Parker *et al.*, 1991.
Hormidium. Kützing sp.[= *Hormidiopsis* Heering]	Klaveness and Rueness, 1986.
H. subtile (Kützing) Heering	Akiyama, 1967, 1968; Pankow *et al.*, 1987, 1991.
Raphidium nivale Lagerh. forma *minor* (= *Elakatothrix* Kützing)	Gain, 1911a.
? R. pyrenogerum Chodat	Fritsch, 1912b.
Raphidonemopsis sessilis Deason	Broady, 1979b.

cont.

Appendix 1 (cont.)

Microsporaceae	
Microspora stagnorum (Kützing) Lagerheim in Heering [syn.: *Conferva stagnorum* Kützing, *Ulothrix stagnorum* Kützing]	Fukushima, 1968; Broady, 1979a; Hirano, 1979, 1983.
**M. tumidula* Hanzen	Cameron, 1966; Luscinska and Kyc, 1993.
cf. Microspora sp.	Curl and Becker, 1970.
Prasiolales	
Prasiolaceae	
**Prasiococcus calcarius* (J. B. Petersen) Vischer	Longton and Holdgate, 1979; Broady, 1979a, 1981a, b, 1982a, 1983, 1984a, 1986, 1987a, 1989c; Fumanti *et al.*, 1993; Ling, 1996; Ling and Seppelt, 1998.
Prasiola antarctica Kützing	West and West, 1911; Fritsch, 1912.
**P. calophylla* (Carm.) Meneghini	Corte, 1962; Broady, 1987a, 1989b, 1989c; Ling and Seppelt, 1998.
**P. crispa* (Lightfoot) Meneghini	West and West, 1911; Fritsch, 1912a, b; Schofield and Ahmadjian, 1972; Seaburg *et al.*, 1979; Broady, 1979a, 1979b, 1984a, 1986, 1987a, 1989c; Engelskjøn, 1981; Smith, 1984c; Ryan *et al.*, 1989; Pankow *et al.*, 1987, 1991; Ling and Seppelt, 1998. Wille, 1902; Gain, 1911b; Vialow and Sdobnikova, 1961; Corte, 1962; Rudolph, 1963; Cameron, 1966; Baker, 1967; Kol, 1968b, 1973; Parker *et al.*, 1972; Longton and Holdgate, 1979; Klaveness and Rueness, 1986; Fumanti *et al.*, 1995; Ling, 1996.
**P. crispa* ssp. *antarctica* (Kützing) Knebel	Ugolini and Starkey, 1966; Hirano, 1983; Jacob *et al.*, 1992.
P. crispa var. *antarctica* (Kützing) Knebel	Kobayashi, 1967.
**P. crispa* var. *antarctica* forma *antarctica* (Kützing) Knebel	Wagner and Zaneveld, 1988.
P. crispa ssp. *antarctica* forma *antarctica* (Kützing) Knebel	Kobayashi, 1967.
P. crispa var. *aspera* West and West	West and West, 1911.
**P. fluviatilis* (Sommer.) Areschoug	Parker *et al.*, 1972; Broady, 1984a.
P. fluviatilis forma *antarctica* Wille	Wille, 1924.
Prasiola Meneghini sp.	Cameron, 1966.
P. tesselata Kützing	Parker *et al.*, 1972.
**P.* uniseriate filaments	Broady, 1987a.
Schizogonium murale	Cameron, 1966; Ling and Seppelt, 1998.
Schizogonium sp.	Ling and Seppelt, 1998.

cont.

Appendix 1 (cont.)

Chaetophorales	
Chaetophoraceae	
Chaetophoroideae	
Stigeoclonium Kützing sp.	Bardin *et al.*, 1971; Priddle and Belcher, 1982.
*Unidentified Chaetophorales sp.	Broady, 1984a.
Ulvelloideae	
Protoderma brownii Fritsch	Fritsch, 1912b; Wille, 1924.
Leptosiroideae	
Coccobotrys mucosus Broady and Ingerfeld	Broady and Ingerfeld, 1993.
**cf. Coccobotrys* Chodat sp.	Broady, 1987a, 1989c; Ryan *et al.*, 1989.
Desmococcus endolithicus Broady and Ingerfeld	Broady and Ingerfeld, 1993.
D. olivaceus (Pers. *ex* Ach.) Laundon	Broady, 1981; Broady and Ingerfeld, 1993; Ling and Seppelt, 1998.
**cf. Desmococcus* sp.	Broady, 1981, 1982a, 1986, 1989b; Smith, 1984c.
**D. vulgaris* Brand	Broady, 1979a, 1979b, 1989a, 1989c; Fumanti *et al.*, 1993; Starmach, 1995.
Diplosphaera mucosa Broady	Broady, 1982c; Pankow *et al.*, 1991.
Diplosphaera Bialosuknia *em.* Vischer sp.	Broady and Smith, 1994.
Dilabifilum prostratum Broady and Ingerfeld	Broady and Ingerfeld, 1993.
Gongrosira Kützing *sensu* Bristol sp. (= *Leptosira* Borzi)	Parker *et al.*, 1972.
G. terricola Bristol	Broady, 1979a, 1979b; Ling and Seppelt, 1998.
Hazenia Bold	Broady, 1979a.
Pleurococcus antarcticus West and West	West and West, 1911; Fritsch, 1912; Wille, 1924; Corte, 1962; Thomas, 1965; Fumanti *et al.*, 1995, 1997.
P. antarcticus forma *filamentosa* Fritsch	Fritsch, 1912; Wille, 1924.
P. antarcticus forma *minor* Fritsch	Fritsch, 1912; Wille, 1924; Corte, 1962.
P. antarcticus forma *robusta* West and West	West and West, 1911; Fritsch, 1912; Wille, 1924; Hirano, 1979, 1983.
P. antarcticus forma *simplex* West and West	Fritsch, 1912; Wille, 1924.
P. antarcticus forma *stellata* Fritsch	Fritsch, 1912; Wille, 1924.
P. antarcticus forma *typica* Fritsch	Fritsch, 1912; Wille, 1924; Corte, 1962.
P. dissectus (Kützing) Nägeli [syn.: *Protococcus dissectus* Kützing]	West and West, 1911; Fritsch, 1912; Wille, 1924; Corte, 1962.
P. frigidus West and West	West and West, 1911; Fritsch, 1912; Wille, 1924.

cont.

Appendix 1 (cont.)

P. koettlitzi Fritsch	Fritsch, 1912a.
P. pachydermus Lagerheim	West and West, 1911; Wille, 1924.
P. pachydermus forma *stipitata* West and West	West and West, 1911; Wille, 1924.
Pleurococcus Menegh. sp.(= *Prasiolopsis* Vischer)	Thomas, 1965; Engelskjøn, 1986.
P. tectorum var. *antarctica* Wille	Wille, 1924; Fumanti *et al.*, 1995.
P. vulgaris Meneghini	Fritsch, 1912.
P. vulgaris var. *cohaerens* Wittr.	Gain, 1911a; Kol, 1972.
**Protococcus grevillei* (= *Apatococcus* Brand *em* Geitler)	Cameron, 1966; Cameron *et al.*, 1970.
**P. nivalis*	Cameron,1966.
Pseudopleurococcus printzii Vischer	Akiyama, 1967, 1968.
Chaetosphaeridiaceae	
**Oligochaetophora simplex* West	Seaburg *et al.*, 1979.
Chaetosphaeridium sp.	Priddle and Belcher, 1982.
Oedogoniales	
Oedogoniaceae	
Oedogonium Link sp.	Priddle and Belcher, 1981, 1982; Oguni *et al.*, 1989.
Ulvophyceae	
Ulotrichales	
Ulotrichaceae	
Monostroma sp.	Parker *et al.*, 1972.
Trichosarcina mucosa (Broady) Chappell and O`Kelly [basio.: *Pseudoschizomeris mucosa* Broady]	Broady, 1982c, 1986; Chappell and O`Kelly, 1991; Ling and Seppelt, 1998.
**Ulothrix flacca* (Dillw.) Thur.	Cameron, 1966.
U. flaccida Kützing	Gain, 1911b; Wille, 1924.
**U. implexa* (Kützing) Kützing	West and West, 1911; Wille, 1924; Wagner and Zaneveld, 1988; Ling and Seppelt, 1998.
**U.* Kützing sp.	Fukushima, 1959; Cameron, 1966; Parker *et al.*, 1972; Longton and Holdgate, 1979; Engelskjon, 1981, 1986; Priddle and Belcher, 1982; Klaveness and Rueness, 1986; Broady, 1987a, 1989c; Luscinska and Kyc, 1993; Broady and Smith, 1994; Ling, 1996; Ling and Seppelt, 1998.

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Appendix 1 (cont.)

U. subtilis Kützing	Fritsch, 1912b.
U. subtilis var. *tenerrima* (Kützing) Kirchner	Gain, 1911a.
U. subtilis var. *tenerrima* forma *antarctica* Wille	Gain, 1911a; Lavrenko, 1967.
U. subtilis var. *variabilis* (Kützing) Kirchner	West and West, 1911; Fritsch, 1912; Wille, 1924.
U. subtilissima Raben. in Heering	Hirano, 1979.
U. tenerrima Kützing in Starmach	Hirano, 1979; Pankow *et al.*, 1991.
U. tenerrima forma *antarctica* West and West	West and West, 1911; Wille, 1924.
U. variabilis Kützing in Prescott	Hirano, 1979, 1983; Luscinska and Kyc, 1993.
U. verrucosa Lokhorst [syn. *Chlorhormidium mucosum* J. B. Petersen in Starmach]	Hirano, 1979
U. zonata (Weber *et* Mohr) Kützing	Broady, 1979a; Ling and Seppelt, 1998.
**Urospora penicilliformis* (Roth) Areschoug	Wagner and Zaneveld, 1988.
**U.* sp.	Broady, 1984a.
Uronema sp.	Pandey *et al.*, 1995; Ling and Seppelt, 1998.
Unidentified alga from Ulotrichales	Luscinska and Kyc, 1993.
Unidentified filamentous algae	Ohtani, 1986; Akiyama *et al.*, 1991; Luscinska and Kyc, 1993.
Pleurastrales	
Pleurastraceae	
Trebouxia cf. impressa Ahmadjian	Aoki *et al.*, 1998.
T. incrustata Ahmadjian and Gärtner	Aoki *et al.*, 1998.
**Trebouxia* Puymaly sp.	Schofield and Ahmadjian, 1972; Parker *et al.*, 1972; Broady, 1979a; Friedmann, 1980, 1982; Meyer *et al.*, 1988; Broady and Smith, 1994; Ling and Seppelt, 1998.
Microthamnion kuetzingianum Nägeli	Broady, 1979a.
M. strictissimum Rabenhorst	Broady, 1979a.
Charophyceae	
Klebsormidiales	
Klebsormidiaceae	
Chlorhormidium dissectum (Chodat) Fott [= *Klebsormidium dissectum* (Gay) Ettl and Gärtner]	Broady, 1979a.
C. flaccidum (Kützing) Fott [= *Klebsormidium flaccidum* (Kützing) Silva, Mattox and Blackwell]	Broady, 1979a, 1979b; Starmach, 1995.
C. flaccidum var. A[= *Klebsormidium flaccidum* var 1]	Broady, 1979a; Ling and Seppelt, 1998.

cont.

Appendix 1 (cont.)

C. mucosum J. B. Petersen in Starmach	Hirano, 1979.
Hormiscia flaccida var. *nivalis* De Wild. (= *Klebsormidium* Silva, Mattox *et* Blackwell)	Wildeman, 1935.
**Klebsormidium subtilissimum* (Raben.) Silva, Mattox and Blackwell	Seaburg *et al.*, 1979; Broady, 1984a.
Koliella helvetica (Kol) Hindák (= *Raphidonema* Lagerheim)	Akiyama, 1967, 1968, 1979.
K. cf. tatrae (Kol in Györffy) Hindák	Klaveness and Rueness, 1986; Pankow *et al.*, 1991.
Raphidonema antarctica Kol	Saag, 1979; Kol, 1972; Pankow *et al.*, 1991.
R. helvetica Kol	Ling, 1996; Ling and Seppelt, 1998.
**R. nivale* Lagerheim	Fritsch, 1912b; Wildeman, 1935; Holm-Hansen, 1964; Corte, 1966; Fogg, 1967; Akiyama, 1977; Seaburg *et al.*, 1979; Broady, 1984a; Ling, 1996; Ling and Seppelt, 1998.
R. nivale forma *minor* Wille	Gain, 1911a; Wille, 1924.
R. pyrenoidifera var. *elongata* Broady	Broady, 1982c.
R. sempervirens (Chodat) Hindák	Broady, 1982c, 1986; Ling and Seppelt, 1998.
**R.* Lagerheim sp.	Broady, 1984a, 1987a; Broady and Smith, 1994.
R. tatrae Kol	Ling, 1996; Ling and Seppelt, 1998.
**Stichococcus bacillaris* Nägeli [syn.: *St. minor* Nägeli, *St. membranaefaciens* Chodat, *St. dubius* Chodat, *St. bacillaris* Nägeli *sens. strict.*, *St. chloranthus* Raths, *St. pallescens* Chodat, *St. coniocybes* Letellier, *St. nivalis* Chodat, *St. viridis* Nakano]	Gain, 1911a; Wille, 1924; Wildeman, 1935; Corte, 1962, 1966; Holm-Hansen, 1964; Cameron, 1966; Akiyama, 1967, 1968, 1977; Longton and Holdgate, 1979; Seaburg *et al.*, 1979; Broady, 1979a, 1979b, 1984a, 1989; Pankow *et al.*, 1987, 1991; Fumanti *et al.*, 1993; Ling, 1996; Fumanti *et al.*, 1997; Ling and Seppelt, 1998.
S. bacillaris forma *major* (Nägeli) Roth	Gain, 1911a.
S. bacillaris forma *minor* Nägeli	Gain, 1911a.
S. exiguus Gern.	Akiyama, 1967, 1968.
**S. flaccidus* (Kützing) Gay [= *Klebsormidium flaccidum* (Kützing) Silva, Mattox and Blackwell]	Cameron, 1966; Parker *et al.*, 1972.
S. minutus Grintzesco *et* Peterfi	Broady, 1979a; Pankow *et al.*, 1991; Ling, 1996; Ling and Seppelt, 1998.
**S. nivalis* Chodat [syn.: *S.bacillaris* var. *genuinus* f. *minor* Nägeli, f. *major* (Nägeli) Roth]	Kol, 1968b, 1973; Saag, 1979; Wagner and Zaneveld, 1988; Pankow *et al.*, 1991.

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Appendix 1 (cont.)

*S. Nägeli sp.	Flint and Stout, 1960; Broady, 1981c, 1986, 1987a; Engelskjøn, 1986; Ohtani and Kanda, 1987; Meyer *et al.*, 1988; Ryan *et al.* 1989; Akiyama *et al.*, 1991; Broady and Smith,1994.
*S. *subtilis* (Kützing) Klercker [= *Klebsormidium subtile* (Kützing) Tracanna and Tell]	Cameron, 1966.
Zygnemaphyceae	
Zygnematales	
Mesotaeniaceae	
Ancylonema nordenskioldii Berggren	Gain, 1911a; Wille, 1924; Saag, 1979; Pankow *et al.*, 1991.
Cylindrocystis brebissonii Meneghini	Parker *et al.*, 1972; Luscinska and Kyc, 1993.
C. brebissonii forma *cryophila*	Kol, 1972.
C. brebissonii var. *minor* West and West	Broady, 1979a; Luscinska and Kyc, 1993.
C. brebissonii forma *turgida*	Luscinska and Kyc, 1993.
C. crassa De Bary	Gain, 1911b; Fritsch, 1912b; Wille, 1924; Lavrenko, 1967; Broady, 1979a; Priddle and Belcher, 1982; Pankow *et al.*, 1991; Luscinska and Kyc, 1993.
C. Meneghini *ex* De Bary sp.	Broady, 1979b; Broady and Smith, 1994.
Gonatozygon brebissonii De Bary	Lucinska and Kyc, 1993.
G. De Bary sp.	Priddle and Belcher, 1982.
Mesotaenium de greyi Turner	Luscinska and Kyc, 1993.
M. berggrenii (Wittr.) Lagerheim	Kol, 1972
M. berggrenii forma	Ling and Seppelt, 1990, 1998; Ling, 1996.
M. endlicherianum Nägeli	Fritsch, 1912b.
M. macrococcum (Kützing) Roy *et* Biss	Luscinska and Kyc, 1993.
M. Nägeli sp.	Longton and Holdgate, 1979.
Netrium oblongum var. *cylindricum* (West *et* West) Kossinski	Pankow *et al.*, 1991.
Netrium (Nägeli) Itzigsohn and Rothe sp.	Broady, 1979a.
Desmidiaceae	
**Actinotaenium cucurbita* (Brébisson in Ralfs) Teiling [syn.: *Cosmarium cucurbita* Brébisson in Ralfs]	Saag, 1979; Broady, 1982b, 1986, 1987a; Ohtani, 1986; Pankow *et al.*, 1987,1991; Akiyama *et al.*, 1991; Fumanti *et al.*, 1993; Luscinska and Kyc, 1993; Ling and Seppelt, 1998.
A. cucurbita var. attenuatum West [syn. *Cosmarium cucurbita* var. *attenuatum* West]	Lavrenko, 1967; Hirano, 1979; Pankow *et al.*, 1991.

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Appendix 1 (cont.)

A. cucurbita var.rotundatum Krieger *et* Gerloff [syn. *Cosmarium cucurbita* var. *rotundatum* (Krieger) Krieger and Gerloff]	Akiyama, 1967, 1968; Hirano, 1979, 1983; Pankow *et al.*, 1991.
A. curtum (Brébisson) Teiling [syn.: *Cosmarium curtum* (Brébisson) Ralfs., *Penium curtum* Bréb., *Calocylindrus curtus* (Brébisson) Kirchner, further synonymy given by Krieger and Gerloff (1969)]	Broady, 1979a.
Closterium moniliferum (Bory) Ehrenberg *ex* Ralfs	Starmach, 1995.
C. parvulum var. *parvulum* Nägeli	Brook and Williamson, 1983.
C. Nitzsch *ex* Ralfs sp.	Priddle and Belcher, 1982.
Cosmarium antarcticum Gain	Gain, 1911b; Wille, 1924.
C. asphaerosporum var. *strigosum* Nordstedt	Lavrenko, 1967; Pankow *et al.*, 1991.
C. binum Nordstedt	Brook and Williamson, 1983.
C. bioculatum var. *depressum* (Sch.) Schmidle in Krieger and Gerloff	Hirano, 1979.
C. clepsydra Nordstedt	Ohtani, 1986.
C. clepsydra var. *depressum* Hirano	Hirano, 1979.
C. clepsydra var. *dissimile* (Racib.) Krieger and Gerloff	Hirano, 1979, 1983; Oguni *et. al.*, 1989.
C. clepsydra var. *granulatum* Hirano	Hirano, 1979.
C. clepsydra var. *undulatum* Hirano	Hirano, 1979.
C. crenatum Ralfs *et* Ralfs	Gain, 1911b; Wille, 1924; Luscinska and Kyc, 1993.
C. cucurbita Brébisson in West and West	Hirano, 1979.
C. decussare Brook and Williamson	Brook and Williamson, 1983.
C. granatum Brébisson *ex* Ralfs	Pankow *et al.*, 1991
C. laeve var. *laeve* Rabenhorst	Priddle and Belcher, 1982; Brook and Williamson, 1983; Pankow *et al.*, 1987, 1991; Starmach, 1995.
C. laeve var. *laeve* forma *majus* Borge in West *et* West	Komárek and Ruzicka, 1966; Saag, 1979; Pankow *et al.*, 1987, 1991.
C. laeve var. *majus* Borge in West *et* West	Pankow *et al.*, 1987.
C. margaritatum (Lund.) Roy and Biss.	Brook and Williamson, 1983.
C. microsphinctum Nordstedt	Luscinska and Kyc, 1993.
C. notabile De Bary [syn.: *Cosmarium notabile* f. *minor* Wille, *Penium notabile* (De Bary) Gay, further synonymy given by Krieger and Gerloff (1965)]	Broady, 1979a.
C. pachydermum Lund.	Luscinska and Kyc, 1993.
C. parvulum Brébisson [syn.: *Cosmarium obcuneatum* West and West, *Actinotaenium obcuneatum* (West) Teiling]	Broady, 1979a.

cont.

Appendix 1 (cont.)

C. phaseolus var. *minus* (Boldt) Krieger and Gerloff	Hirano, 1979.
C. polygonum (Nägeli) Arch.	Brook and Williamson, 1983.
C. praemorsum Brébisson	Pankow *et al.*, 1991.
C. regnellii Wille	Priddle and Belcher, 1982.
C. sexangulare var. *minus* Roy and Biss.	Brook and Williamson, 1983.
C. Corda sp.	Fukushima, 1959; Parker *et al.*, 1972; Akiyama, 1979; Broady, 1979a; Priddle and Belcher, 1982.
C. speciosum Lünd.	Luscinska and Kyc, 1993.
C. speciosum forma	Luscinska and Kyc. 1993.
C. subcrenatum Hantzsch in West and West	Hirano, 1979, 1983.
C. subspeciosum Nordstedt	Luscinska and Kyc, 1993.
C. subspeciosum var. *validus* Nordstedt	Priddle and Belcher, 1982.
C. subtumidum Nordstedt in Wittrock *et* Nordstedt	Lavrenko, 1967; Pankow *et al.*, 1991.
C. undulatum Corda	Gain, 1911b; Wille, 1924.
C. undulatum var. *minutum* Wittrock [syn.: *Cosmarium crenatum* var. *alpinum* Racib., *C. crenatum* var. *minutum* Racib., *C. undulatum* f. *minor* West *et* West, *C. alpinum* (Racib.) De Toni, *C blanjinense* Pevalek, *C. undulatum* f *minima* Gutwinski, *Euastrum undulatum* f. *minor* Gay]	Broady, 1979a.
Cosmoastrum dilatum (Ehr.) Pal-Mordv.(= *Staurastrum* Meyen)	Lucinska and Kyc, 1993.
C. punctulatum (Brébisson) Pal-Mordv.	Lucinska and Kyc, 1993.
Penium Brébisson sp.	Fritsch, 1912; Wille, 1924; Fukushima, 1959; Priddle and Belcher, 1982.
Pleurotaeniopsis pseudoconnata (Nordst.) Lagerheim	Gain, 1911b; Wille, 1924.
Staurastrum acarides Nordst.	Luscinska and Kyc, 1993.
S. coarctatum Brébisson	Pankow *et al.*, 1991.
S. disputatum West and West [syn.: *S. dilatum* var. *insignis* Racib.]	Parker *et al*, 1972.
S. muticum Brébisson	Broady, 1979a.
S. punctulatum Brébisson	Priddle and Belcher, 1982.
S. punctulatum fac. *quadriradiata*	Brook and Williamson, 1983.
S. sp.	Oguni *et al.*, 1989; Ling and Seppelt, 1998.
Zygnemataceae	
Spirogyra Link sp.	Priddle and Belcher, 1982; Hawes, 1988.

cont.

Appendix 1 (cont.)

Mougeotia (Agardh) Wittrock sp.	Fritsch, 1912b; Priddle and Belcher, 1982; Hawes, 1989.
Zygnema Agardh sp.	Fritsch, 1912b; Wille, 1924; Priddle and Belcher, 1982; Hawes, 1989, 1990; Luscinska and Kyc, 1993.

Xanthophyta
Xanthophyceae
Mischococcales
Pleurochloridaceae

Chloridella neglecta Pascher	Broady, 1979a.
Chlorocloster minimus Pascher	Kol, 1968b.
C. pyreniger var. minor Starmach	Starmach, 1995.
C. terrestris Pascher	Starmach, 1995.
Ellipsoidion cf. perminimum Pascher	Broady, 1979a, 1979b.
E. perminimum var. cryophila Kol	Kol and Flint, 1968.
E. solitare (Geitler) Pascher	Parker *et al.*, 1972.
Ellipsoidion sp Pascher	Parker *et al.*, 1972; Broady, 1979a; Ling, 1996; Ling and Seppelt, 1998.
**Monodus coccomyxa* Pascher	Seaburg *et al.*, 1979.
M. chodatii	Fumanti *et al.*, 1993.
M. fusiformis Starmach	Starmach, 1995.
Nephrodiella semilunaris Pascher	Fumanti *et al.*, 1993.
Pleurochloris commutata Pascher	Starmach, 1995.
P. polychloris Pascher	Starmach, 1995.
Trachychloron ellipsoideum Pascher	Parker *et al.*, 1972.
Unidentified genus of the family Pleurochloridaceae	Broady, 1979a.

Centritractaceae

**Bumilleriopsis* Printz	Flint and Stout, 1960; Ling and Seppelt, 1998.
B. brevis (Gerneck) Printz	Cameron and Benoit, 1970.

Characiopsidaceae

Characiopsis Borzi	Broady, 1979a.
C. borziana Lemm.	Starmach, 1995.

Botrydiopsidaceae

**Botrydiopsis* Borzi	Broady, 1979b, 1984a, 1987a, 1989a, c; Smith, 1984a, b; Broady and Smith 1994; Ling and Seppelt, 1998.

cont.

Appendix 1 (cont.)

**B. antarctica* Kol	Kol, 1970; Pankow *et al.*, 1991.
B. arhiza Borzi [syn.: *B. turfosa* Pascher]	Akiyama, 1968; Broady, 1986.
B. constricta Broady	Broady, 1976, 1979a, 1979b.
B. intercedens Pascher [syn.: *B. alpina* Vischer]	Broady, 1986.
Botryochloridaceae	
Botryochloris minima Pascher	Starmach, 1995
**cf. Chlorellidium* Vischer and Pascher in Pascher	Broady, 1987a; Broady and Smith, 1994.
C. tetrabotrys Vischer and Pascher in Vischer	Broady, 1986.
Ilsteria lobata Pascher	Starmach, 1995.
Gloeobotrydaceae	
Gloeobotrys Pascher sp.	Broady, 1979a, 1979b.
G. terrestris Reisigl	Broady, 1979a.
Tribonematales	
Tribonemataceae	
**Bumilleria* Borzi sp.	Holm-Hansen, 1964.
C. glacialis Kützing [syn.: *C. glacialoides* Wolle]	Gain, 1911b.
Xanthonema antarcticum (Broady) Ettl and Gärtner [= *Hormidiospora verrucosa* Vinatzer]	Broady, 1979a.
**X. bristoliana* (Pascher) Silva [syn. *Heterothrix bristoliana* Pascher]	Holm-Hansen, 1964; Saag, 1979; Seaburg *et al.*, 1979; Pankow *et al.*, 1991.
X. debile (Visher) Silva [syn.. *H. debilis* Vischer]	Broady, 1979a, 1979b; Ling and Seppelt, 1998.
X. exile (Klebs) Silva [syn. *H. exilis* Pascher, *Bumilleria exilis* Klebs]	Akiyama, 1967, 1968; Broady, 1979a, 1979b; Fumanti *et al.*, 1993.
X. hormidioides (Visher) Silva	Broady *et al.*, 1997.
**X. montanum* (Visher) Silva [syn. *H. cf. montana* Visher]	Broady, 1984a.
X. sessile (Vinatzer) Ettl and Gärtner	Broady *et al.*, 1997.
**Xanthonema* Silva sp.[syn. *Heterothrix* Pascher sp.]	Flint and Stout, 1960; Broady, 1986, 1987a; Broady and Smith, 1994; Broady *et al.*, 1997.
Heterotrichella gracilis Reisigl	Broady, 1979a; Fumanti *et al.*, 1993.
Tribonema bombycinum (Ag.) Derbés and Solier [syn.: *Conferva bombycina* Ag.]	Fritsch, 1912a, b; Corte, 1962.
T. elegans Pascher	Broady, 1982b.

cont.

Appendix 1 (cont.)

T. glacialis Kützing	Lavrenko, 1967.
T. microchloron Ettl	Ling and Seppelt, 1998.
Tribonema Derbés *et* Solier sp.	Priddle and Belcher, 1982; Pankow *et al.*, 1991; Broady and Smith, 1994.
T. ulotrichoides Pascher	Luscinska and Kyc, 1993.
T. vulgare Pascher [syn.: *T. bombycinum* f. *tenuis* Hazen, *Conferva bombycina* f. *tenuis* Collins]	Broady, 1979a; Starmach, 1995.
Heterococcaceae	
Fremya Dangeard sp.	Ling and Seppelt, 1998.
Monocilia viridis Gern. (= *Heteropedia* Pascher)	Akiyama, 1967, 1968.
M. komarkovae Nyg.	Pankow *et al.*, 1987.
Heterococcus caespitosus Vischer	Darling *et al.*, 1987.
H. chodatii Vischer	Broady, 1979a; Starmach, 1995.
H. endolithicus Darling *et* Friedmann	Darling *et al.*, 1987.
H. filiformis Pitschm.	Ling and Seppelt, 1998.
**H. moniliformis* Vischer	Seaburg *et al.*, 1979; Wagner and Zaneveld, 1988.
H. pleurococcoides Pitschmann	Darling *et al.*, 1987.
H. protonematoides Vischer	Darling *et al.*, 1987.
**H.* Chodat sp.	Flint and Stout, 1960; Friedmann, 1982; Broady, 1984a, 1986; Broady and Smith, 1994; Ling and Seppelt, 1998.
H. tectiformis Pitschmann	O`Kelly, 1989.
Vaucheriales	
Vaucheriaceae	
Vaucheria De Candolle sp.	Wille, 1924.

Appendix 2. S-Plus commands for analysis of morphological and isozyme data.

```
ls()

data <- read.table("a:name of worksheet.csv", sep = ",", header = T, row.names =
NULL)

data

win.graph()

data.dist <- dist(t(as.matrix(data[,2:final column])), metric = "binary")

data.dist

dist.clus <- hclust(data.dist, method = "average")

plclust(dist.clus, hang = -1, labels = names (data[,2:final column]))

win.printer(file = "a:/clus.wmf" , h=8.27, w=11.59, format = "placeable")

plclust(dist.clus, hang = -1, labels = names(data[,2:final column]))

dev.off()
```

Appendix 3. Characters and character [a]states used in phenetic analysis of [b]*Botrydiopsis* and [c]*Chlorellidium.*

A. Vegetative Cell

1. Size
1.1. 10-20μm
1.2. 21-30μm
1.3. >31μm

2. Shape
2.1. Spherical
2.2. Ellipsoidal
2.3. Spherical to ellipsoidal

3. Cell wall
3.1. Thick
3.2. Thin
3.3. Smooth
3.4. Verrucose

4. Lens shaped thickening
4.1. Present
4.2. Absent

B. Chloroplast

5. Shape
5.1. Spindle
5.2. Discoidal
5.3. Ellipsoidal
5.4. Polygonal

6. Number (adult cell)
6.1. < 20
6.2. > 20

C. Pyrenoid

7. Occurrence
7.1. Present
7.2. Absent

8. Type
8.1. Immersed
8.2. Bulged

9. Number
9.1. One
9.2. two

D. Sporangium

10. Size (μm)
10.1. ~20
10.2. 20-30
10.3. 30-40
10.4. >40

11. Shape
11.1. Spherical
11.2. Ellipsoidal
11.3. Spherical to ellipsoidal

12. Cell wall
12.1. Smooth
12.2. Verrucose

13. Number of spores
13.1. ~20
13.2. >20

E. Zoospore

14. Shape
14.1. Spherical
14.2. Ellipsoidal
14.3. Pyriform
14.4. Amoeboid

15. Chloroplast numbers
15.1. One
15.2. Two

16. Stigma
16.1. Present
16.2. Absent

17. Position of stigma
17.1. Anterior
17.2. Median
17.3. Posterior

18. Number of flagella in LM
18.1. One
18.2. Two

Appendix 3 (cont.)

19. Length of long flagellum (μm)
19.1. ~5
19.2. ~10

F. Aplanospore

20. Shape
20.1. Spherical
20.2. Ellipsoidal

21. Number of chloroplast
21.1. One to two
21.2. three to four
21.3. > four

G. Mucilage sheath

22.1. Present
22.2. Absent

H. Constriction / budding of adult cells in *Botrydiopsis*

23.1. Frequent in both young and old cultures.
23.2. Rare in both young and old cultures.
23.3. Present (rare) in young culture but absent in old culture.
23.4. Absent in both young and old cultures.

I. Ability to cluster

24.1. No clustering
24.2. Clustering

---

[a]All characters were scored as '1/O/NA', where 1 indicates presence of character, 0 indicates absence and NA indicates not applicable because feature not present in the strain.
[b] See Appendix 4 for morphological data matrix of 27 strains in *Botrydiopsis.*
[c] See Appendix 5 for morphological data matrix of eight strains in *Chlorellidium.*

Appendix 4. Morphological data matrix of 27 strains in *Botrydiopsis*.

[a]Characters	Strains												
	645	894	829	801	G41/6	WI/1	908	724	895	896	897	886	G12/1
1.1	1	1	1	1	1	1	1	1	1	1	1	1	1
1.2	1	1	1	1	1	1	1	1	1	1	1	1	1
1.3	1	1	1	1	1	1	0	1	1	1	1	1	1
2.1	0	0	0	0	0	0	1	0	0	0	0	0	0
2.2	0	0	0	0	0	0	0	0	0	0	0	0	0
2.3	1	1	1	1	1	1	0	1	1	1	1	1	1
3.1	1	1	1	1	1	1	1	1	1	1	1	1	1
3.2	0	0	0	0	0	0	0	0	0	0	0	0	0
3.3	1	1	1	1	1	1	1	1	1	1	1	1	1
3.4	0	0	0	0	0	0	0	0	0	0	0	0	0
4.1	1	1	1	1	1	1	1	1	1	1	1	1	1
4.2	0	0	0	0	0	0	0	0	0	0	0	0	0
5.1	1	1	1	1	1	1	1	1	1	1	1	1	1
5.2	1	1	1	1	1	1	0	1	1	1	1	1	1
5.3	0	0	0	0	0	0	0	0	0	0	0	0	0
5.4	1	1	1	1	1	1	0	1	1	1	1	1	1
6.1	0	0	0	0	0	0	0	0	0	0	0	0	0
6.2	1	1	1	1	1	1	1	1	1	1	1	1	1
7.1	0	0	0	0	0	0	1	0	0	0	0	0	0
7.2	1	1	1	1	1	1	0	1	1	1	1	1	1
8.1	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	NA
8.2	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA
9.1	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA
9.2	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	NA
10.1	0	0	0	0	0	0	0	0	0	0	0	0	0
10.2	0	0	0	0	0	0	1	0	0	0	0	0	0
10.3	1	1	1	1	1	1	0	1	1	1	1	1	1
10.4	0	0	0	0	0	0	0	0	0	0	0	0	0
11.1	0	0	0	0	0	0	1	0	0	0	0	0	0
11.2	0	0	0	0	0	0	0	0	0	0	0	0	0
11.3	1	1	1	1	1	1	0	1	1	1	1	1	1

[a]See Appendix 3 for explanation of character states.

Appendix 4 (cont.)

[a]Characters	Strains												
	645	894	829	801	G41/6	WI/1	908	724	895	896	897	886	G12/1
12.1	1	1	1	1	1	1	1	1	1	1	1	1	1
12.2	0	0	0	0	0	0	0	0	0	0	0	0	0
13.1	0	0	0	0	0	0	0	0	0	0	0	0	0
13.2	1	1	1	1	1	1	1	1	1	1	1	1	1
14.1	1	1	1	1	1	1	1	1	1	1	1	1	1
14.2	0	0	0	0	0	0	0	0	0	0	0	0	0
14.3	1	1	1	1	1	1	1	1	1	1	1	1	1
14.4	1	1	1	1	1	1	0	1	1	1	1	1	1
15.1	1	1	1	1	1	1	1	1	1	1	1	1	1
15.2	0	0	0	0	0	0	0	1	1	1	1	1	1
16.1	1	1	1	1	1	1	1	1	1	1	1	1	1
16.2	0	0	0	0	0	0	0	0	0	0	0	0	0
17.1	1	1	1	1	1	1	1	1	1	1	1	1	1
17.2	0	0	0	0	0	0	0	0	0	0	0	0	0
17.3	0	0	0	0	0	0	0	0	0	0	0	0	0
18.1	0	0	0	0	0	0	0	0	0	0	0	0	0
18.2	1	1	1	1	1	1	1	1	1	1	1	1	1
19.1	0	0	0	0	0	0	0	1	1	1	1	1	1
19.2	1	1	1	1	1	1	1	0	0	0	0	0	0
20.2	1	1	1	1	1	1	1	1	1	1	1	1	1
20.2	1	1	1	1	1	1	0	1	1	1	1	1	1
21.1	1	1	1	1	1	1	1	1	1	1	1	1	1
21.2	1	1	1	1	1	1	0	1	1	1	1	1	1
21.3	0	0	0	0	0	0	0	0	0	0	0	0	0
22.1	1	1	1	1	1	1	0	0	0	0	0	0	0
22.2	0	0	0	0	0	0	1	0	0	0	0	0	0
23.1	1	1	1	1	1	1	0	0	0	0	0	0	0
23.2	0	0	0	0	0	0	0	0	0	0	0	0	0
23.3	0	0	0	0	0	0	0	1	1	1	1	1	1
23.4	0	0	0	0	0	0	1	0	0	0	0	0	0
25.1	1	1	1	1	1	1	1	0	0	0	0	0	0
25.2	0	0	0	0	0	0	0	1	1	1	1	1	1

Appendix 4 (cont.)

[a]Characters	Strains													
	485	G94/1	836	837	G9/8	G19/2	G24/8	G27/1	G31/	G99/2	877	638	864	G27/2
1.1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1.2	0	1	1	1	1	1	1	1	1	1	1	1	1	1
1.3	0	0	1	0	0	0	0	0	0	0	1	1	0	0
2.1	0	0	0	0	0	0	0	0	0	0	1	1	1	1
2.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.3	1	1	1	1	1	1	1	1	1	1	0	0	0	0
3.1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.3	1	1	0	1	1	1	1	1	1	1	1	1	1	1
3.4	0	0	1	0	0	0	0	0	0	0	0	0	0	0
4.1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
4.2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
5.1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
5.2	0	0	0	1	1	1	1	1	1	1	1	1	1	1
5.3	1	1	1	0	0	0	0	0	0	0	0	0	0	0
5.4	1	1	1	1	1	1	1	1	1	1	0	0	1	1
6.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7.1	0	0	0	1	1	1	1	1	1	1	1	1	1	1
7.2	1	1	1	0	0	0	0	0	0	0	0	0	0	0
8.1	NA	NA	NA	0	0	0	0	0	0	0	1	1	1	1
8.2	NA	NA	NA	1	1	1	1	1	1	1	0	0	0	0
9.1	NA	NA	NA	1	1	1	1	1	1	1	1	1	1	1
9.2	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0
10.1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
10.2	0	0	0	0	0	0	0	0	0	0	0	0	1	1
10.3	0	0	0	1	1	1	1	1	1	1	1	1	0	0
10.4	0	1	1	0	0	0	0	0	0	0	0	0	0	0
11.1	0	1	1	0	0	0	0	0	0	0	1	1	1	1
11.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11.3	1	0	0	1	1	1	1	1	1	1	0	0	0	0

Appendix 4 (cont.)

[a]Characters	Strains													
	485	G94/1	836	837	G9/8	G19/2	G24/8	G27/1	G31/5	G99/2	877	638	864	G27/2
12.1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
12.2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
13.1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
13.2	0	0	0	0	0	0	0	0	0	0	1	1	0	0
14.1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
14.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14.3	1	0	1	1	1	1	1	1	1	1	1	1	1	1
14.4	0	0	0	0	0	0	0	0	0	0	0	0	1	1
15.1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
15.2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
16.1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17.1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19.1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
19.2	0	0	0	0	0	0	0	0	0	0	1	1	0	0
20.2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20.2	1	1	1	1	1	1	1	1	1	1	0	0	1	1
21.1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
21.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21.3	0	0	1	1	1	1	1	1	1	1	1	1	1	1
22.1	0	0	0	1	1	1	1	1	1	1	0	0	1	1
22.2	1	1	1	0	0	0	0	0	0	0	1	1	0	0
23.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23.2	1	0	1	1	1	1	1	1	1	1	0	0	1	1
23.3	0	1	0	0	0	0	0	0	0	0	0	0	0	0
23.4	0	0	0	0	0	0	0	0	0	0	1	1	0	0
25.1	0	0	1	0	0	0	0	0	0	0	1	1	0	0
25.2	1	1	0	1	1	1	1	1	1	1	1	1	1	1

Appendix 5. Morphological data matrix of eight strains in *Chlorellidium*.

[a]Characters	Strains							
	597	757	758	898	G28/7	G31/6	785	871
1.1	1	1	1	1	1	1	1	1
1.2	1	1	1	1	1	1	1	1
1.3	1	1	1	1	1	1	0	0
2.1	0	0	0	0	0	0	0	0
2.2	0	0	0	0	0	0	0	0
2.3	1	1	1	1	1	1	1	1
3.1	1	1	1	1	1	1	1	1
3.2	0	0	0	0	0	0	0	0
3.3	1	1	1	1	1	1	1	1
3.4	0	0	0	0	0	0	0	0
4.1	1	1	1	1	1	1	1	1
4.2	0	0	0	0	0	0	0	0
5.1	1	1	1	1	1	1	0	0
5.2	1	1	1	1	1	1	1	1
5.3	0	0	0	0	0	0	0	0
5.4	1	1	1	1	1	1	1	1
6.1	0	0	0	0	0	0	0	0
6.2	1	1	1	1	1	1	1	1
7.1	0	0	0	0	0	0	1	1
7.2	1	1	1	1	1	1	0	0
8.1	NA	NA	NA	NA	NA	NA	0	0
8.2	NA	NA	NA	NA	NA	NA	1	1
9.1	NA	NA	NA	NA	NA	NA	1	1
9.2	NA	NA	NA	NA	NA	NA	0	0
10.1	1	1	1	1	1	1	1	1
10.2	1	1	1	1	1	1	1	1
10.3	1	1	1	1	1	1	0	0
10.4	0	0	0	0	0	0	0	0
11.1	0	0	0	0	0	0	0	0
11.2	0	0	0	0	0	0	0	0
11.3	1	1	1	1	1	1	1	1

[a]See Appendix 3 for explanation of character states.

Appendix 5 (cont.)

[a]Characters	Strains							
	597	757	758	898	G28/7	G31/6	785	871
12.1	1	1	1	1	1	1	1	1
12.2	0	0	0	0	0	0	0	0
13.1	1	1	1	1	1	1	0	0
13.2	0	0	0	0	0	0	1	1
14.1	1	1	1	1	1	1	1	1
14.2	0	0	0	0	0	0	0	0
14.3	1	1	1	1	1	1	1	1
14.4	1	1	1	1	1	1	1	1
15.1	1	1	1	1	1	1	1	1
15.2	0	0	0	0	0	0	0	0
16.1	1	1	1	1	1	1	1	1
16.2	0	0	0	0	0	0	0	0
17.1	1	1	1	1	1	1	1	1
17.2	0	0	0	0	0	0	0	0
17.3	0	0	0	0	0	0	0	0
18.1	0	0	0	0	0	0	0	0
18.2	1	1	1	1	1	1	1	1
19.1	0	0	0	0	0	0	0	0
19.2	1	1	1	1	1	1	1	1
20.2	1	1	1	1	1	1	1	1
20.2	1	1	1	1	1	1	1	1
21.1	0	0	0	0	0	0	0	0
21.2	0	0	0	0	0	0	0	0
21.3	1	1	1	1	1	1	1	1
22.1	1	1	1	1	1	1	1	1
22.2	0	0	0	0	0	0	0	0
23.1	0	0	0	0	0	0	0	0
23.2	0	0	0	0	0	0	0	0
23.3	0	0	0	0	0	0	0	0
23.4	0	0	0	0	0	0	0	0
25.1	0	0	0	0	0	0	0	0
25.2	1	1	1	1	1	1	1	1